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ERRATA.

Page 23, 20 lines from end, for " *Eucalyptua* " read " *Eucalyptus* "

Page 84, insert after line 18 " moved per harvest. In September, 3·1 per cent. of the total ripe pods were infested "

Page 113, 17 lines from end, for " five " read " seven "

Page 139, the third line of the paragraph starting " The figures obtained " should read " only as regards thickness and pores, but also chemically. These variations make it "

Page 169, 13 lines from end, for " tota " read " total "

Page 205, line 11, for " nunber " read " number "

Page 343, line 10, for " *Xanthoroë* " read " *Xanthorhoë* "

Page 380, the first reference for *Diatraea saccharalis* (F.) should read " *Phalaena saccharalis* Fabricius, 1794, Skrift. af Naturh.-Selsk., Copenhagen, **3** (2), p. 64, Pl. vii, fig. 1 "

Page 424, line 13, for " femora " read " tibia "

Page 462, line 31, for " A. L. Moutia " read " L. A. Moutia "

Page 635, Table X, line 17 of col. 1, for " *corniclatus* " read " *corniculatus* "

Page 691, line 7 of 2nd para, for " Mr. J. F. D. Thomas " read " Mr. F. J. D. Thomas "

COMMONWEALTH INSTITUTE OF ENTOMOLOGY.

BULLETIN
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Vol. 42. 1951.

THE BEHAVIOUR AND MORTALITY OF *ANOPHELES MACULATUS* AND *CULEX FATIGANS* IN EXPERIMENTAL HUTS TREATED WITH DDT AND BHC.*

By R. H. Wharton.

Institute for Medical Research, Kuala Lumpur.

(Plate I.)

The experiments recorded in this paper, briefly reported earlier (Wharton & Reid, 1950), were undertaken to estimate the relative values of DDT (dichloro-diphenyl-trichloroethane) and BHC (benzene hexachloride) as residual insecticides against *Anopheles maculatus* Theo. in Malaya. Reports from many parts of the world have indicated the value of DDT, but Thomson (1947, 1949) found it relatively ineffective against *A. gambiae* Giles in Africa; BHC on the other hand was most effective. Thomson suggested that the irritant contact effect of DDT could nullify *its* toxicity under certain circumstances and he found that the mortality of *A. gambiae* entering huts treated with DDT was negligible, whereas BHC killed 100 per cent. during thirteen weeks.

These results confirmed the belief expressed by Reid (1948) that careful investigations of both insecticides were necessary before widespread house-spraying should be attempted as a measure to control *A. maculatus*, the principal vector of malaria in Malaya. Wallace (1948) had already shown that *A. maculatus* entered rooms treated with DDT or BHC and fed normally, but that DDT reduced the morning catch (5 a.m. to dawn) to zero for many weeks. No information on the mortality of *A. maculatus* leaving such rooms was available. In order to obtain this information, the method of trap huts employed by Thomson appeared to offer the best solution.

It was necessary to determine whether *A. maculatus* females would enter experimental trap huts, and whether, having entered, they could be recovered in window

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* This work was carried out with the aid of a grant from the Colonial Welfare and Development Fund.

traps the following morning. Since *A. maculatus* does not rest in houses by day, the second point was all important. Thomson (1948), in his studies on *A. gambiae*, used simple mud and thatch huts; Anophelines entered through small fissures and chinks in the eaves, but when attempting to leave were attracted to the light from a single window opening. The design of hut needed modification for Malayan conditions—the numbers of *A. maculatus* available for the investigation were small and it was considered doubtful if hungry Anophelines would enter through adventitious openings in palm thatch. Furthermore, it was desired to construct smooth-walled huts which could be easily but efficiently searched, so that all Anophelines remaining within the huts could be recovered.

Preliminary observations furnished some details of the normal behaviour of *A. maculatus* and these necessitated changes in the original design and position of the huts. Other aspects of the behaviour of *A. maculatus* females did not become apparent until the insecticide experiments were in progress. Before describing the results of DDT and BHC applications, all these points must be considered.

Large numbers of Culicines, mainly *Culex fatigans* Wied. entered the huts and afforded an opportunity of observing their behaviour and mortality under similar conditions.

Construction of Huts.

Three experimental huts were constructed, in which the floor and framework were made of wood and the roof and walls of palm thatch (attap). The actual dimensions were 8 ft. long, 5 ft. wide, 7 ft. 6 ins. high at the eaves, the floor being raised 1 ft. 6 ins. from the ground (Pl. I, fig. 1). Each hut was lined with a smooth, opaque brown paper (seisal-craft) to prevent light from entering, and to make it as easy as possible to find mosquitos; the ceiling was also made of the same substance. The door, situated at one corner, was screened internally by two overlapping curtains of black cloth supported by vertical spring curtain rods (Pl. I, fig. 2). A detachable trap was installed* at one end, over the single window opening. The trap was similar in design to Thomson's (1948), but the frame was wooden, the funnel opening $\frac{1}{2}$–$\frac{3}{4}$ in. in diameter and about 1 in. from the front wall of the trap. The complete funnel was arranged so that it sloped gradually upwards (Pl. I, fig. 3) to facilitate the exit of mosquitos which tend to fly upwards (Reid, 1951).

Owing to the complete paper lining and the tight fitting curtains over the door, each hut was sealed when the light trap was in position and mosquitos had no means of entrance. After trying various types of entrance without success, a solution was found by fitting simple louvres (Pl. I, fig. 2), two series for each hut. Each series consisted of a number of blackened half-inch boards, 2 ft. 6 ins. long by 6 ins. wide, placed at an angle of 30 degrees with the vertical, and $1\frac{1}{4}$ ins. apart. The overall dimensions of a series were 2 ft. 6 ins. wide by 5 ft. 6 ins. high and provided eighteen openings each 2 ft. 6 ins. long and $1\frac{1}{4}$ ins. wide. The louvres did allow some light to enter but, when viewed from the interior during the hours of darkness, the trap in the window was the only obvious source of light. Earlier unsuccessful types of entrance which had been tried were light proof but also prevented *A. maculatus* from entering the huts.

A simple roller blind was attached to the inside of each louvre series to reduce the number of mosquitos which might escape back through the louvres. When the blinds were lowered at 5 a.m., the only possible exit was into the window trap. For the second insecticide application a further refinement was added in the form of an open mosquito net cage (Louvre trap, Pl. I, figs. 1, 2) which could be fixed over a portion of the louvre surface.

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* Wharton and Reid (1950) refer to this trap as a light trap.

Site of Experiments.

A rubber estate in the Tampin district of Negri Sembilan was chosen for the experiments where, prior to the Japanese occupation of Malaya, malaria control was carried out by oiling the numerous streams and ravines on the estate. At the time of the experiments, the estate labour force was issued with suppressive proguanil (paludrine), there was no oiling of streams, etc., and the breeding of *A. maculatus* was unchecked (fig. 1). Adult *Anopheles*, caught by the use of a human bait net trap (Gater, 1935) indicated that reasonable numbers were entering the area of the labourers' lines (H.B.T. fig. 2) at the time when observations commenced.

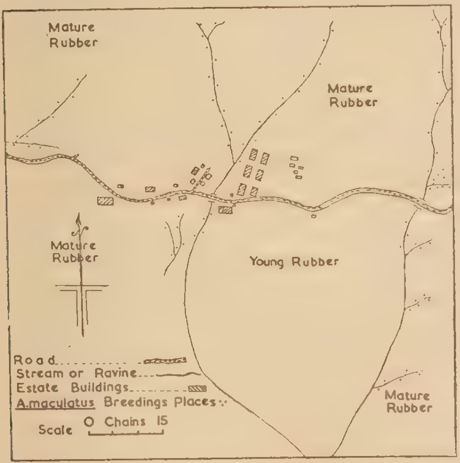
Mature Rubber
Mature Rubber
Mature Rubber
Young Rubber
Mature Rubber
Road
Stream or Ravine
Estate Buildings
A.maculatus Breedings Places
Scale 0 Chains 15

Fig. 1.—Sketch of estate area showing *A. maculatus* breeding places in relation to labourers' lines.

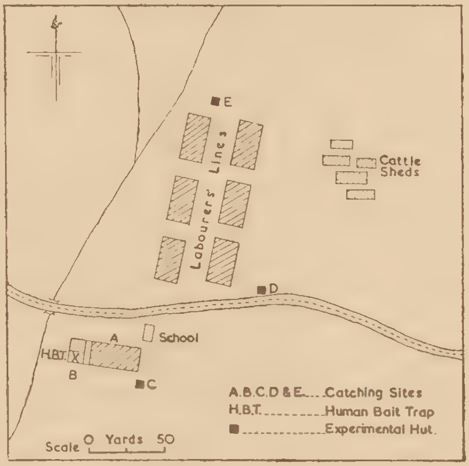
E
Labourers' Lines
Cattle Sheds
D
A
School
H.B.T.
B
C
A.B.C.D & E. Catching Sites
H.B.T. Human Bait Trap
Experimental Hut.
Scale 0 Yards 50

Fig. 2.—Sketch of labourers' lines area showing catching sites and position of experimental huts.

Initially, the huts were placed equidistant from each other at the points of a triangle within the lines area (site A, fig. 2). The position proved unsatisfactory and more favourable sites were chosen after considering the results of several nights observations during which collectors caught mosquitos on their legs at various places around the lines area. The results, shown in Table I, suggested that sites C and D were better than the others, with little to choose between B and E. B was discarded since it was close to the human bait net trap and could possibly have affected the catch there. The final positions of the experimental huts, C, D, and E are shown in fig. 2.

TABLE I.

Numbers of *A. maculatus* caught on legs at different sites around lines area.

Trapping Site	A	B	C	D	E	H.B.T.*
Number of Nights	2	5	6	4	4	6
Total caught	6	31	77	62	26	30
Average per Night	3·0	6·2	12·8	15·5	6·5	5·0

* Human bait net trap results for comparison.

It was noted that the more favourable catching stations were situated at the periphery of the lines area; presumably mosquitos were intercepted on their way into the main feeding area.

Procedure during Observations.

When the huts were operated normally, an estate labourer entered each hut at 7 p.m. and remained until 5 a.m. the following morning. A member of the staff checked the movements of the labourers, fixed the traps in position and arranged a sheet carefully on the floor of each hut before 7 p.m., and lowered the blinds over the louvres at 5 a.m. The huts were searched at 8 a.m., the window traps taken down and returned to the laboratory where the mosquitos from the huts and the traps were identified. Any *A. maculatus* taken alive were placed in lamp globe cages in the insectarium, provided with water and raisins, and the subsequent mortality after 8, 24 and 48 hours recorded. Mosquitos were recorded as blood fed or unfed, and alive or dead, moribund specimens being regarded as alive until all movement ceased. The approximate number of *A. maculatus* in the window trap at midnight, 5, 6 and 7 a.m. was noted.

Observations on Culicines were limited to recording initial mortality, distribution, and the proportion captured which had fed. Subsequent mortality of *C. fatigans* taken alive was observed for a short period during the second experiment. Male Culicines were excluded from the results.

It was found necessary to take precautions against the loss of dead or affected mosquitos from inside the huts. Spiders, ants, cockroaches and small lizards were a constant menace and could not be eliminated completely. In the second experiment, the hut supports were treated once weekly with a sticky banding substance (bird lime) and sodium silicofluoride was dusted through the attap walls every week. In order to form an estimate of the number of mosquitos lost in this way, and to check the effectiveness of the precautions, dead mosquitos gummed to small strips of filter paper were distributed over the floor and the number removed during the night recorded.

In an endeavour to gain a more accurate picture of the feeding activity of *A. maculatus* a number of " leg trapping " experiments were carried out. In these, two collectors were stationed in each hut from 6 p.m. to 6 a.m. with instructions to catch all mosquitos attempting to bite them ; on these nights the labourer was not present. The human bait net trap was operated throughout the whole period of observations.

Behaviour of Anophelines in untreated Huts.

Although other species of *Anopheles* were present in large numbers around the labourers' lines, only *A. maculatus* was found consistently in trap hut collections. The " other *Anopheles* " formed a large proportion of the total captured in the human bait net trap, and attempted to bite collectors sitting in the open. In the preliminary observations* 136 Anophelines were recovered from the huts of which 120 (90 per cent.) were *A. maculatus*. Over the same period, 432 Anophelines were trapped by the human bait net trap, 130 (30 per cent.) being *A. maculatus*. Comparable figures, from leg catches in the open, were 311 total Anophelines and 134 (43 per cent.) *A. maculatus*. The almost complete absence of " other *Anopheles* " from trap hut collections was surprising—the conditions did not differ greatly from those in the human bait net trap and it was to be expected that the proportions of the different species captured by the two methods would have been more or less the same. Leg trapping within the huts showed that the other species were not entering the huts, probably deterred by the more difficult means of entrance. The fact that *A. maculatus* entered the huts illustrates, once again, its relative preference for human blood in Malaya. The other species were largely *A. philippinensis* Ludl. and *A. aconitus* Dön., with some *A. hyrcanus* (Pall.) and *A. vagus* Dön.

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* These exclude three nights' results from one hut when an electric light was tried in the hut. Eleven " other *Anopheles* " were caught, none blood fed, and presumably attracted by the light.

Behaviour of A. maculatus.

(i) Recovery from hut.

The majority recovered had taken blood and were found in the window trap at 8 a.m. Results from the untreated control hut during the second insecticide experiment showed that 91 per cent. of the total were blood fed and 80 per cent. were in the window trap. The use of louvre traps in this period indicated that many of those which entered escaped back through the louvres. The louvre trap catch was assumed to represent one fourth of the total escaping in this way since the trap covered one fourth of the total louvre surface. From this assumption it was possible to estimate the total entry and subsequent behaviour of *A. maculatus* in an untreated hut. These estimates have been shown in Table II.

TABLE II.

Number of *A. maculatus* recovered compared to number estimated to have entered an untreated hut.

(Control hut results, Experiment 2.)

	Window trap	Louvre trap	Hut	Total	Total recovered as percentage of total estimated to have entered.
Recovered	380	80	20	480	*67*
Percentage of Total ..	*79*	*17*	*4*	*100*	
Estimation	380	320	20	720	
Percentage of Total ..	*53*	*44*	*3*	*100*	

Thus the window trap caught only 53 per cent. of the number estimated to have entered, 44 per cent. escaped via the louvres, and 3 per cent. remained in the hut (showing clearly that *A. maculatus* did not rest in the huts by day). Only 67 per cent. of the number estimated to have entered were recovered.

The leg trapping experiments provided further evidence that more *A. maculatus* entered untreated huts than could be accounted for by normal trapping. In 12 nights' observation, the average catch per night on the legs was 37, compared to an average of 12 from 27 normal observations, both series being obtained during a four week period at the beginning of the second insecticide experiment. The available *A. maculatus* population was about the same for the two sets of observations, for the human bait net trap caught an average of 24 per night, whilst leg trapping was in progress, and 27 per night during normal trapping. The figures are not strictly comparable, however, since there were two collectors in the huts for the leg trapping and only one estate labourer at normal times. Ribbands (1949) has shown that in West Africa there exists a close correlation between the number of men used as bait and the number of Anophelines attracted.

(ii) Time of departure from the hut.

The approximate numbers of *A. maculatus* in the window trap at midnight, 5, 6 and 7 a.m., together with the recorded number at 8 a.m. are shown in Table III. (Again the results have been taken from the control hut records of the second experiment.) Other Anophelines represented less than 1 per cent. of the total.

TABLE III.

Approximate number of *A. maculatus* in window trap at midnight and early morning.

	Midnight	5 a.m.	6 a.m.	7 a.m.	8 a.m.
Numbers ..	46	114	166	203	239
Per cent. ..	*19*	*48*	*70*	*85*	*100*

Counts were made by torchlight up to 6 a.m. Constant movement within the traps made counting difficult. It was clear that many *A. maculatus* left the hut well before dawn. According to the counts, 19 per cent. of the total recovered in the trap had entered by midnight and almost 50 per cent. by 5 a.m. Dawn broke shortly before 6 a.m. and the sun rose at about 7 a.m. during the period of these observations.

(iii) Feeding activity in the huts.

Information on the time of feeding was obtained from the leg trapping experiments. In 24 all-night observations, 417 *A. maculatus* were captured attempting to feed. Of these, 45 (11 per cent.) were taken between 6 and 9 p.m.,226 (54 per cent.) from 9 to 12, 79 (19 per cent.) from 12 to 3 a.m., and 67 (16 per cent.) from 3 to 6 a.m. The period of greatest activity, then, was between 9 p.m. and midnight, agreeing with past observations by Wallace (1948).

(iv) Comment.

Wallace (1939, 1948) recorded many *A. maculatus* captured in labourers' lines from 5 a.m. to dawn, resting on the walls. These records, together with the earlier results obtained in the huts (of 52 recovered only three were in the window trap by 5 a.m.) led to the belief that *A. maculatus* remained quiescent after feeding until dawn, when they were attracted to the window and caught in the window trap. The insecticide trials were commenced with this belief, but it became apparent that many *A. maculatus* left well before dawn, and that many were not attracted by the light from the window but made their way out through the louvres. Reid (1951) has recently indicated that *A. vagus* is more strongly attracted to light than *A. maculatus* ; species evidently vary considerably in this respect, and it seems possible that other factors, such as fresh air draughts, may have some influence in determining their exit from darkened huts or rooms.

Behaviour of Culicines in untreated Huts.

Culex fatigans formed about 95 per cent. of the Culicines found in trap hut collections. In contrast to *A. maculatus*, the majority remained in the huts and were found resting on the walls at 8 a.m. The proportion in the window trap varied from week to week within fairly wide limits from a maximum of 60 per cent. to a minimum of 10 per cent., but the average for all observations was 28 per cent. The biting rate was high, for 80 per cent. of those recovered contained blood. Males were also present but are not included in the results.

DDT and BHC as residual Insecticides in experimental Huts.

Preliminary observations indicated that it should be possible to determine the effect of an application of residual insecticides inside the huts. Although the number of *A. maculatus* was not large, it was thought that sufficient would be recovered in the traps and the hut to show the overall effect. The changes in available *A. maculatus* population before and during the insecticide experiments are indicated in fig. 3. The graph is based on human bait net trap catches, the trap operating for about 20 nights in each month.

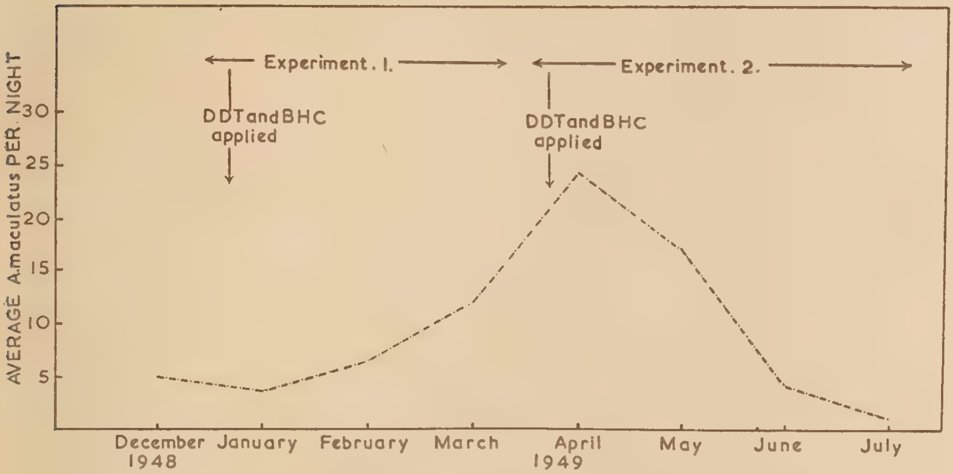

Fig. 3.—Variations in the population of adult female *A. maculatus* during the period of the observations in relation to the times of the insecticide experiments. Average number captured per night in the human bait net trap.

It was not possible to identify all collections of Culicines from the huts, but *C. fatigans* was always the dominant species. Consequently the effect of insecticides on all Culicines recovered was assumed to represent the effect on *C. fatigans* alone.

Application and formulation of insecticides.

Both insecticides were applied with a brush as 10 per cent. suspensions of water dispersible powders—DDT (Stafford Allen's 33 per cent. w.d.p.) at the rate of 100 mg. DDT/sq. ft. for the first, and 200 mg. DDT/sq. ft. for the second experiment—BHC (I.C.I.'s " Gammexane ") P530 at the rate of 20 mg. γ isomer/sq. ft. for the first, and P.520 at 40 mg. γ isomer/sq. ft. for the second experiment. Both BHC powders contained 6.5 per cent. γ isomer. Two huts were treated in each experiment, one with DDT, the other with BHC, the insecticides being applied to all the internal surfaces except the door curtain, louvres and floor for the first, and including the door curtain for the second experiment. The third hut was left untreated as the control.

Experiment 1.

DDT at 100 mg./sq. ft. and BHC at 20 mg. γ isomer/sq. ft.

The results of this application were most disappointing in that very few *A. maculatus* were recovered from either of the treated huts.

(i) Effect on *A. maculatus*.

Table IV summarises the results.

TABLE IV.

Mortality of *A. maculatus* recovered from experimental huts treated with 100 mg. DDT/sq. ft. and 20 mg. γ BHC/sq. ft.

Hut	Numbers	Before treatment	Weeks after treatment				
			1	2	3	5	8
DDT	Total	5	1	3	1	6	26
	Found dead	0	1	2	0	3	3
	Dead after 24 hours	0	1	3	1	3	6
BHC	Total	5	1	2	3	16	27
	Found dead	0	1	0	0	1	2
	Dead after 24 hours	0	1	1	0	3*	2
Control	Total	0	7	19	4	16	12
	Found dead	0	0	0	0	0	2
	Dead after 24 hours	0	0	1	1	0	3
Number of observations		5	5	5	5	5	5

* 4 lost eaten by ants in laboratory.

DDT.—No *A. maculatus* recovered during the first three weeks lived for 24 hours after capture although two were alive in the window trap when found. In the fifth week all those in the window trap (three) were alive and survived for 24 hours, but the remainder (three) were dead on the floor. By the eighth week the application appeared to have little effect.

BHC.—As early as the second week, one *A. maculatus* survived for 24 hours and in the third week there was no mortality in the three recovered.

(ii) Effect on Culicines (mainly *C. fatigans*).

The mortality of all Culicines recovered is summarised in Table V.

TABLE V.

Mortality of Culicines (mainly *C. fatigans*) in experimental huts treated with 100 mg. DDT/sq. ft. and 20 mg. γ BHC/sq. ft.

		Before treatment	Weeks after treatment				
			1	2	3	5	8
DDT	Total recovered	222	133	105	48	213	89
	Number dead	0	9	5	1	5	0
	Per cent. dead	*0*	*7*	*5*	*2*	*2*	*0*
BHC	Total recovered	112	31	53	35	159	73
	Number dead	0	20	11	2	5	0
	Per cent. dead	*0*	*65*	*21*	*6*	*3*	*0*
Control	Total recovered	112	192	117	96	282	67
	Number dead	0	0	0	2	1	0

DDT.—The treatment had very little effect on mortality, a few being found dead on the floor of the hut.

BHC.—The initial mortality of 65 per cent. was not maintained and the application was relatively ineffective by the third week.

The irritant quality of DDT was well shown by the reactions of Culicines. Table VI shows the percentages of the total numbers recovered which were in the window traps of the three huts.

TABLE VI.

Effect of treatment on Culicines. Number in window trap as percentage oı total recovered.

	Before treatment	Weeks after treatment				
		1	2	3	5	8
DDT	31	79	61	67	78	69
BHC	19	39	25	37	21	25
Control	10	15	20	27	22	27

(iii) Comment.

The experiment was not a success because of the low numbers of *A. maculatus* recovered. This was in part due to the small population available at the time, but other factors were considered to have been responsible for the drop in numbers recovered from the DDT and BHC huts immediately after they were treated. An investigation of these factors brought to light defects in technique and an increase in knowledge of the behaviour of *A. maculatus*.

Leg trapping results during the fourth week after treatment suggested that more *A. maculatus* were entering the huts than could be accounted for by normal trapping, and this was more pronounced in the treated huts (Table VII).

TABLE VII.

Average catches per night of *A. maculatus* from experimental huts. Third and fifth week—normal trapping; fourth week—leg trapping.

Period	Number of Nights	Average *A. maculatus* per night		
		DDT hut	BHC hut	Control hut
Third week	5	0·2	0·6	0·8
Fourth week	4	3·5	6·5	4·0
Fifth week	5	1·2	3·2	3·2

These results led to the trial of a simple trap over a portion of the louvres and the consequent proof that *A. maculatus* did escape through them.

Observations in the huts at night revealed the presence of spiders, small lizards, ants and cockroaches and suggested that dead or affected mosquitos were being removed before the huts were searched. Proof of this was obtained by gumming dead mosquitos to filter paper and leaving them on the floors of the huts. From 111 dead mosquitos placed in the DDT hut at 9 p.m. in this way, only 62 were found at 8 a.m. Similar results were recorded from the control hut. Steps were then taken to reduce these losses for the second experiment.

One further possibility should be mentioned, the distinct musty odour of BHC was evident some distance from the hut for two weeks after treatment, and still noticeable when standing beside the hut during the third week. It was thought that BHC may have acted as a repellent to both *A. maculatus* and Culicines.

Further comments on this experiment will be found on page 15.

EXPERIMENT 2.

DDT at 200 mg./sq. ft. and BHC at 40 mg. γ isomer/sq. ft.

The old paper walls and ceilings were replaced by fresh paper before re-treating. " Gammexane P520 " was applied in the BHC hut and precautions were taken against losses of dead mosquitos ; louvre traps were in operation, two in the DDT hut and one each in the BHC and control huts. The experiment was timed to coincide with the normal seasonal rise in *A. maculatus* population (see fig. 3). After the first 8 to 10 weeks, the Anopheline population decreased and finally the observations were terminated 16 weeks after treatment when very few *A. maculatus* were being recovered.

The complete results for *A. maculatus* and Culicines (mainly *C. fatigans*) have been summarised by weeks and are shown as an Appendix (pages 19, 20).

(i) Effect on *A. maculatus.*

Mortality.

Both insecticides produced a considerable mortality with BHC outstanding. In the DDT hut dead *A. maculatus* were found on the floor up to thirteen weeks after treatment and formed a large proportion (34 per cent.) of the total catch. The majority in the traps were alive when found at 8 a.m., but few survived for 24 hours in the insectarium. As shown in Table VIII the mortality of blood fed *A. maculatus* in the window trap at 8 a.m. was 34 per cent. rising to 74 per cent. after 24 hours, while 76 per cent. of those unfed were found dead at 8 a.m. Mortality among *A. maculatus* from the control hut was so high beyond 24 hours that it was impossible to determine further insecticidal effect. The reason for this high mortality is not known, although transport from the hut site to the laboratory, a distance of some five miles, may have been partly responsible.

TABLE VIII.

A. maculatus found in window traps (16 weeks observations). Mortality when found and after 24 hours in the insectarium.

	Blood fed			Unfed		
	DDT	BHC	Control	DDT	BHC	Control
Total	152	112	342	38	107	38
Found dead	51	70	1	29	105	6
Per cent.	*34*	*63*	*0·3*	*76*	*97*	*16*
Dead after 24 hours ..	113	107	43	38	107	33
Per cent.	*74*	*96*	*13*	*100*	*100*	*87*

Dead *A. maculatus* were found in the BHC hut for ten weeks after treatment—the total found dead in this way was 20 per cent. of the total catch, considerably less than in the DDT hut. All *A. maculatus* in the traps, however, were dead at 8 a.m. for four weeks after treatment, and the majority recorded alive after that were moribund when found. For the sixteen weeks of the experiment only five *A. maculatus* taken alive in the window trap survived for 24 hours (Table VIII)—and these were moribund.

As would be expected, the mortality of mosquitos in the window and louvre traps was approximately the same. The number caught in the louvre traps, however, was surprisingly small, particularly in the DDT hut where it had been thought that irritated mosquitos might escape into the louvres in larger numbers. Estimations of the total number that entered and of mortality (Table IX) did not differ greatly from the actual results obtained.

TABLE IX.

Recovery and mortality of *A. maculatus* from experimental huts with estimates of total entry and mortality.

		Recovered in			Total recovered	Estimated loss*	Estimated total entry
		Window trap	Louvre trap	Hut			
DDT	Number	190	41	119	350	41	391
	Dead after 24 hrs. ..	151	34	118	303	34	337
	Per cent. dead ..	*80*	*83*	*99*	*86·7*	*83*	*86·3*
BHC	Number	219	13	58	290	39	329
	Dead after 24 hrs. ..	214	13	58	285	39	324
	Per cent. dead ..	*98*	*100*	*100*	*98·2*	*100*	*98·6*

* Two louvre traps on DDT hut, one on BHC hut.

It was possible, then, to consider the overall mortalities of *A. maculatus* recovered from the experimental huts. Figs. 4 and 5 show the corrected mortalities (initial and 24 hour) using Abbott's formula (1925) for *A. maculatus* from the DDT and BHC huts respectively.

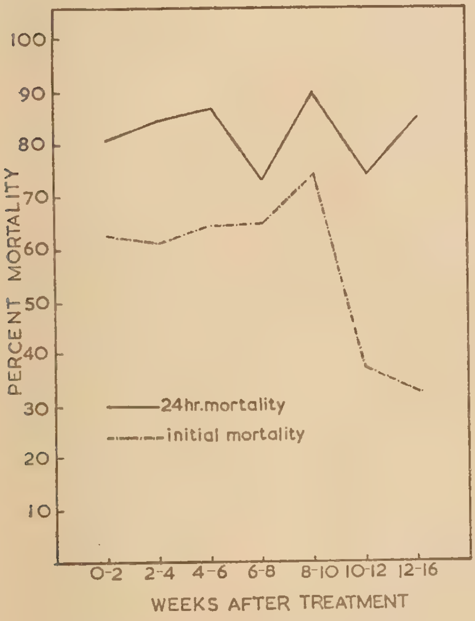

Fig. 4.—Corrected mortality of *A. maculatus* recovered from an experimental hut treated with 200 mg. DDT/sq. ft.

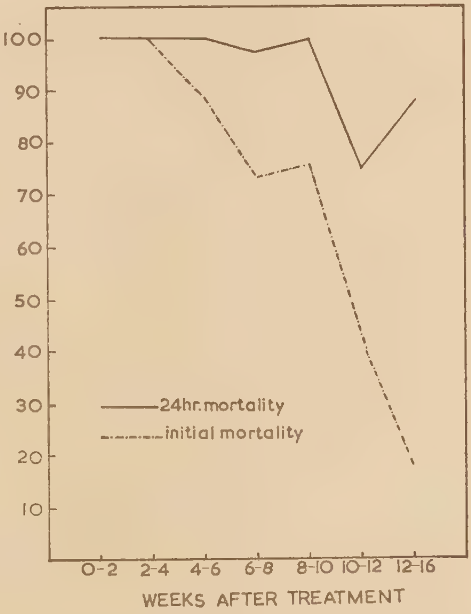

Fig. 5.—Corrected mortality of *A. maculatus* recovered from an experimental hut treated with 40 mg. γ BHC/sq. ft.

For DDT the initial mortality was 63 per cent. and the 24-hour mortality 83 per cent. for the period of the experiment. With BHC the initial mortality dropped from 100 per cent in weeks 1–4, to 19 per cent. in weeks 12–16 but the *effective* mortality (24 hours), almost 100 per cent. for weeks 1–10, was still 88 per cent. for the final four weeks of the experiment.

Feeding.

The insecticide applications did not prevent *A. maculatus* from biting. The percentage blood fed mosquitos in collections from the DDT hut was only slightly lower than comparable figures from the control. BHC on the other hand reduced the biting rate considerably for six weeks after which feeding increased and appeared to be about normal over the final four weeks of observations.

TABLE X.

Percentage *A. maculatus* blood fed in treated and untreated huts.

	Before treatment	Weeks after treatment						
		1–2	3–4	5–6	7–8	9–10	11–12	13–16
DDT	89	87	80	80	77	88	77	67
BHC	96	52	39	48	63	77	67	81
Control ..	85	88	91	91	91	93	87	94

During the first six weeks after treatment 62 blood fed and 87 unfed *A. maculatus* were found in the traps of the BHC hut—an indication of the extent to which females were deviated from their blood meal.

(ii) Effect on Culicines (mainly *C. fatigans*).

Mortality.

Fig. 6 shows the corrected mortality of all Culicines recovered from the treated huts. Clearly Culicines were more resistant than *A. maculatus* to both DDT and BHC.

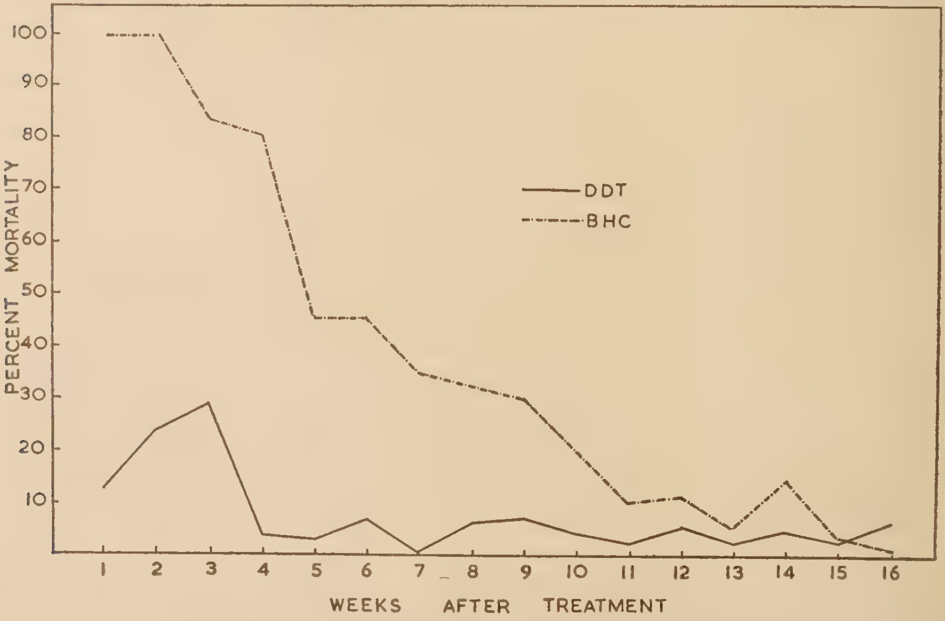

Fig. 6.—Corrected mortality of Culicines (mainly *C. fatigans*) recovered from experimental huts treated with DDT (200 mg. /sq. ft.) and BHC (40 mg. γ isomer /sq. ft.).

As in the first experiment DDT had little effect on mortality. BHC was 100 per cent. effective for two weeks and continued to kill large numbers up to eight weeks after treatment. A check for delayed mortality 24 hours after capture was made in the sixth and subsequent weeks. This mortality was negligible in mosquitos from the control and DDT huts, but contact with BHC produced a delayed mortality up to the tenth week (Table XI).

TABLE XI.

C. fatigans recovered alive from the experimental huts. Subsequent mortality after 24 hours in the insectarium.

Weeks after treatment		BHC						DDT	Control
		6	7	8	9	10	11	6–8	6–11
FED	Number tested ..	30	46	24	34	20	16	77	233
	Dead after 24 hrs. ..	9	7	4	3	6	0	0	3
	Per cent. dead ..	*30*	*15*	*17*	*9*	*30*	*0*	*0*	*1*
UNFED	Number tested ..	50	50	50	37	3	20	65	201
	Dead after 24 hrs. ..	25	9	9	5	0	1	1	8
	Per cent. dead ..	*50*	*18*	*18*	*13*	*0*	*5*	*1*	*4*

Feeding.

Both insecticides reduced the biting rate. The results are summarised in Table XII.

TABLE XII.

Percentage of Culicines blood fed in treated and untreated huts (four-week periods).

	Before treatment	Weeks after treatment			
		1–4	5–8	9–12	13–16
DDT	79	47	46	46	40
BHC	73	28	26	48	55
Control	74	77	82	82	71

Again BHC was more effective than DDT, particularly in the earlier weeks, but in neither hut had feeding returned to normal by the time observations ceased.

Irritant effect.

BHC appeared to parallel DDT in driving Culicines out of the treated huts in this experiment. Table XIII shows that, in both the DDT and BHC hut, 77 per cent. of the Culicines recovered were in the window trap as against 32 per cent. for the control hut (louvre traps results excluded).

TABLE XIII.

Effect of insecticides in driving Culicines out of huts. Number in window trap as percentage of total recovered in window trap and hut (four-week periods).

	Before treatment	Weeks after treatment				Mean post-treatment
		1–4	5–8	9–12	13–16	
DDT	60	85	75	76	73	77
BHC	59	86	82	69	69	77
Control	50	31	25	29	41	32

There was no obvious explanation for the difference in behaviour of Culicines towards the BHC deposit in this experiment.

(iii) Comment.

The overall number of *A. maculatus* captured in the treated huts was considerably lower than the number from the control hut—DDT 350, BHC 290 and control 480. These figures when transformed to estimated entry (on the basis of louvre catches) become DDT 391, BHC 329, and control 720 (Table II). The unexpected low catch in the louvre traps was partly responsible for this difference which is unlikely to have been entirely due to natural fluctuations. Some dead mosquitos were still lost in the huts and this loss is likely to have been greater in the treated huts where many more mosquitos were knocked down, than in the control hut. Approximately 1 in 10 bait mosquitos was removed despite the precautions taken against cockroaches, etc. Spiders seemed to be unaffected by the insecticide applications and it is thought that they were responsible for removing affected mosquitos, though proof could not be obtained.

There remains also the question of BHC repellence. Evidence of this was not clear but was suggested by the occurrence in all observations on *A. maculatus* and Culicines, of an apparent drop in overall catch for a week to two weeks after treatment, while control catches were maintained or increased (Table XIV).

TABLE XIV.

Average catches of mosquitos per night from experimental huts.

		Before treatment	Weeks after treatment			
			1	2	3	4
A. maculatus	DDT	14·2	8·0	4·6	8·6	14·8
	BHC	9·3	5·3	3·7	5·3	10·8
	Control	5·3	12·6	24·3	11·6	8·2
Culicines	DDT	8·6	17·6	16·0	36·3	56·2
	BHC	12·2	5·0	18·0	44·3	84·8
	Control	9·0	19·3	30·0	53·0	48·0

Similar results were obtained in Experiment 1. For further remarks on this subject see the discussion.

From observations on the time of entry into the window traps it was not possible to obtain evidence that *A. maculatus* was irritated by the insecticide applications. The records of numbers in the window traps showed that of the total, the percentage which had entered by midnight were, DDT 26 per cent., BHC 21 per cent., and control 19 per cent. The 5 a.m. figures from all huts were almost the same. These results indicated little change in time of departure from the huts, but more precise methods of recording numbers in the traps would be required to confirm this point.

Only four specimens of Anophelines other than *A. maculatus* were found in the treated huts. One blood fed *A. philippinensis* alive in the window trap of the DDT hut in the eighth week, two unfed *A philippinensis* dead in the BHC window trap in the third and fourth weeks, and one unfed *A. vagus* dead in the BHC hut two weeks after treatment.

Among the Culicines from the experimental huts were a few *Mansonia* species, *M. uniformis* (Theo.) and *M. longipalpis* (Wulp), both vectors of filariasis in some parts of Malaya. Only *M. uniformis* was found in the treated huts, and all specimens taken (8 from the DDT and 6 from the BHC hut) were dead or died within 8 hours of capture. These included specimens from the DDT hut 15 weeks, and from the BHC hut 13 weeks, following the application of the insecticides.

Discussion.

The results demonstrated that *A. maculatus* is susceptible to deposits of DDT and BHC, and agree with those of Thomson (1949) in showing the great effectiveness of BHC as a residual insecticide against mosquitos. In every respect, except persistence, BHC was superior to DDT.

Although the first experiment was not a satisfactory test of either insecticide because of the low numbers of *A. maculatus* recovered, the results did suggest that applications of 100 mg. DDT /sq. ft. and 20 mg. γ BHC /sq. ft. were effective against *A. maculatus* for only five to eight weeks and two to three weeks respectively. This very short effect appears anomalous when compared with the 12 to 16 week period of effectiveness obtained in the second experiment. It does not seem likely that the larger deposits applied in the second experiment would account entirely for this discrepancy in persistence. Part of the explanation may lie in the fact that no reliance can be placed on the records of mosquitos " found dead " on the floor of treated huts in the first experiment. Judging by later observations, mosquitos knocked down by the insecticides could have been removed by spiders, etc. before the huts were searched. The loss could have been of any magnitude and could have occurred throughout the experiment. In the case of DDT, however, the main factor suggesting that the 100 mg. deposit was ineffective after five to eight weeks was the absence of delayed mortality among *A. maculatus* recovered alive. This, though suggestive, was not conclusive since the delayed mortality in the second experiment was variable —normally about 80 per cent., occasionally 100 per cent. and sometimes nil, *e.g.* ten *A. maculatus* were found alive in the window and louvre traps during the eighth week of the second experiment and all were alive after 24 hours. For BHC no such explanation is possible, *A. maculatus* recovered alive from the hut three weeks after the application of 20 mg. γ BHC /sq. ft. behaved normally, whereas no *A. maculatus* from the hut treated with 40 mg. lived for 24 hours until seven weeks after the application.

On the basis of observations on *C. fatigans* this marked difference in effect of the two BHC, but not the DDT, applications was againt evident. A high mortality was recorded for one week only with 20 mg. γ BHC, while the 40 mg. deposit killed 100 per cent. for two weeks and was still lethal several weeks later. Different BHC powders were used, P530 for the first, and P520 for the second experiment. It is possible that the sample of P530 had deteriorated before application to the walls of the hut.

From the above discussion, it is clear that the first experiment must be disregarded in any consideration of the relative merits of DDT and BHC. It is considered that the second experiment was a reliable test of the insecticides, DDT at 200 mg. per sq. ft. and BHC at 40 mg. γ isomer per sq. ft., and conclusions are drawn from this experiment alone.

There was some evidence that BHC acted as a repellent to *A. maculatus* and *C. fatigans*. Relatively large numbers of both species were caught unfed in the window trap, obviously attempting to escape from the BHC treated hut. Reid (1951) found that *A. vagus* adults were repelled at a short distance by a fresh film of BHC and that subsequently many died. The behaviour of *A. maculatus* and *C. fatigans* would suggest that a similar reaction occurred in the hut. The effect was strongest in the first eight weeks following treatment. It appeared also that BHC reduced the number of mosquitos which entered the hut for about two weeks, again suggestive of a repellent effect. There seems no reason to suggest that BHC, like DDT, was irritating to the mosquitos—Cutkomp (1947) found that *A. quadrimaculatus* was not irritated when liberated in BHC treated houses.

The effect of DDT differed from Thomson's results with *A. gambiae*—not only was an appreciable number of *A. maculatus* killed inside the hut, but many of those which escaped into the window trap had picked up a lethal dose. Similar results have been obtained by various workers in the United States using *A. quadrimaculatus*. Metcalf & others (1945) found that 95 to 100 per cent. of the number trapped on their way out of treated houses died in the following 48 hours. Tarzwell & Stierli (1945) observed the mortality over 24 and 48 hours of the same species leaving occupied and unoccupied rooms treated with DDT. These observations were of particular interest since it was found that the effective mortality (48 hours) was reduced considerably by the presence of untreated furnishings in the occupied rooms (mortality 90 per cent. in unoccupied, to 54 per cent. in occupied rooms). Further investigations are necessary to determine whether the conditions of the second experiment, which were well suited to obtaining a high kill, may have given a false impression of the powers of DDT and BHC to kill *A. maculatus* in ordinary dwellings.

C. fatigans and *A. gambiae* appear to react in a similar way to a deposit of DDT. A large proportion of both species rests indoors by day, but when DDT is applied a marked residual irritant, but not lethal, effect is observed, driving them out from their normal resting places. Resistance of *C. fatigans* to DDT deposits has been noted in the past by Ribbands (1947) and Giglioli (1948)—Giglioli found a great reduction in the average *C. fatigans* population of bedrooms following residual DDT treatment but breeding continued in pit latrines nearby. Brown & Williams (1949) after a 21 months DDT spray programme on the island of St. Croix concluded that the use of DDT as a residual spray could not be considered as an effective measure for the eradication of *C. quinquefasciatus* (*fatigans*) but that it reduced the numbers caught in houses by 50 per cent. The present investigation supports these findings and would suggest that the use of DDT as a residual spray will not reduce the overall population of *C. fatigans*, provided suitable out of door resting places are available, but that it will reduce the biting rate of mosquitos entering a treated room. BHC treatment on the other hand should reduce both the overall population and the biting rate.

Laboratory tests against *A. vagus* adults by Reid (1951) confirm the superior residual powers of DDT over BHC for Malayan conditions, but show that a 40 mg. γ BHC deposit still retains some of its toxic effects after 16 weeks. The results recorded here indicate that BHC was still effective against *A. maculatus* (on the baisis of 24 hour mortality) 12 to 16 weeks after application, but do not show the limit of effectiveness for a 40 mg. deposit against this species. There was, however, a considerable loss in toxicity as the BHC deposit aged, shown by the drop in the initial mortalities of *A. maculatus* and particularly *C. fatigans*. The diminution in toxicity of the DDT deposit to *A. maculatus* was much less pronounced. Davidson (1949) and Thomson (1949) indicate that under certain conditions at least 13 to 16 weeks control of Anophelines can be expected from deposits of 10 and 24 mg. γ BHC/sq. ft. respectively. It remains to be seen what are the most satisfactory doses of DDT and BHC under Malayan conditions, and how long they will last.

Summary.

Experimental huts fitted with window traps, of the same basic design as those described by Thomson (1948), modified for Malayan conditions, were used in studying the behaviour and mortality of *A. maculatus* in relation to DDT and BHC. Other species of Anophelines formed a large proportion of the human bait trap catch but rarely entered the huts.

In untreated huts, *A. maculatus* fed at all hours of the night with a peak between 9 p.m. and midnight. The large majority left before 8 a.m. in search of outdoor resting places, at least 50 per cent. before dawn. It appeared that attraction to light was the strongest, but not the only factor influencing exit from the huts.

Large numbers of Culicines (mostly *Culex fatigans*) were recovered. In untreated huts about 80 per cent. were blood fed ; 28 per cent. were in the window traps.

DDT (33 per cent.) and BHC (" Gammexane P530 and P520 ") water dispersible powders were tested.

An application of 100 mg. DDT and 20 mg. γ BHC (P530) per sq. ft. was unsuccessful, due in part to the small numbers of mosquitos recovered. No conclusions are drawn from the results.

DDT at 200 mg. and BHC (P520) at 40 mg. γ isomer per sq. ft. were both effective for at least 12 to 16 weeks against *A. maculatus*. With DDT, 63 per cent. initial, and 83 per cent. 24-hour corrected mortalities were recorded over 16 weeks. With BHC, though the initial mortality dropped from 100 per cent. in weeks 1–4 to 19 per cent. in weeks 12–16, the 24-hour mortality, almost 100 per cent. for weeks 1–10, was still 88 per cent. for weeks 12–16.

DDT apparently had little effect on the feeding of *A. maculatus* ; with BHC there was a reduction in the percentage fed.

DDT was relatively non-lethal to *C. fatigans*, but had a marked irritant effect driving them into the light trap and the biting rate was reduce to 45 per cent. BHC killed all *C. fatigans* which entered for 2 weeks but most of its toxic effects were lost by the eighth week. The biting rate was reduced to 27 per cent. for weeks 1 to 8 and the majority of mosquitos were recovered in the light trap.

Ants, cockroaches, spiders, etc., were found to affect the number of mosquitos recovered and special precautions had to be taken against losses.

The results suggested that BHC when freshly applied acts as a repellent to mosquitos.

Mansonia uniformis was shown to be susceptible to both DDT and BHC residual deposits.

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APPENDIX.

Results of Experiment 2. Experimental huts treated with 200 mg. DDT per sq. ft. and 40 mg γ BHC per sq. ft.

(a) *A. maculatus.*

			Before treat-ment	Weeks after treatment															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Number of observations		6	3	3	3	5	5	5	5	5	5	5	5	5	5	5	5	5
DDT hut	In Hut	Fed Alive ..	0	3	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0
		Dead ..	0	5	8	3	24	16	10	10	6	1	5	2	1	0	0	0	0
		Unfed Alive ..	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
		Dead ..	0	2	0	1	6	3	4	2	0	0	1	0	1	1	0	0	0
	In Traps	Window trap total	73	12	5	15	32	50	12	20	17	9	6	5	1	3	2	1	0
		Louvre trap total..	12	2	1	6	12	2	5	5	4	0	2	0	2	0	0	0	0
		Fed Alive ..	76	7	3	13	19	29	3	9	10	3	5	4	2	1	2	1	0
		Dead ..	0	4	3	4	16	18	5	5	9	4	3	0	1	0	0	0	0
		Unfed Alive ..	9	1	0	3	1	1	0	3	0	0	0	1	0	0	0	0	0
		Dead ..	0	2	0	1	8	4	9	8	2	2	0	0	0	2	0	0	0
BHC hut	In Hut	Fed Alive ..	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
		Dead ..	0	7	3	2	4	4	3	5	3	2	2	0	0	0	0	0	0
		Unfed Alive ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Dead ..	0	1	1	1	9	3	2	2	3	0	0	0	0	0	0	0	0
	In Traps	Window trap total	47	5	6	13	40	61	17	19	18	9	4	6	3	12	3	1	2
		Louvre trap total..	6	3	1	0	1	2	0	3	1	0	0	0	0	0	2	0	0
		Fed Alive ..	51	0	0	0	0	10	0	8	6	2	1	4	1	10	3	0	1
		Dead ..	0	2	2	12	9	20	7	5	7	4	2	1	0	0	1	1	0
		Unfed Alive ..	2	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
		Dead ..	0	6	5	1	32	33	10	9	6	3	0	1	2	1	1	0	1
Control hut	In Hut	Fed Alive ..	1	1	5	0	3	1	3	0	1	1	1	1	0	0	1	1	0
		Dead ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Unfed Alive ..	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
		Dead ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	In Traps	Window trap total	23	33	53	30	30	35	40	41	15	18	17	16	10	14	13	9	6
		Louvre trap total..	8	4	15	5	8	6	10	10	0	1	1	7	3	1	4	4	1
		Fed Alive ..	26	27	65	32	33	39	44	47	14	18	16	18	13	15	14	13	7
		Dead ..	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
		Unfed Alive ..	5	10	2	2	3	2	5	4	0	1	1	4	0	0	3	0	0
		Dead ..	0	0	1	1	1	0	1	0	1	0	1	1	0	0	0	0	0

APPENDIX.

(b) Culicines (mainly *C. fatigans*).

				Before treat-ment	Weeks after treatment															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Number of observations			6	3	3	3	5	5	5	5	5	5	5	5	5	5	5	5	5
DDT hut	In Hut	Fed	Alive ..	19	2	4	9	39	44	85	95	41	50	55	79	25	47	50	34	17
			Dead ..	0	1	1	2	5	1	8	5	4	1	7	5	1	2	0	2	1
		Unfed	Alive ..	0	1	1	0	0	11	6	16	1	2	2	6	1	17	0	9	1
			Dead ..	0	3	1	1	1	0	3	2	5	1	0	1	0	1	0	0	0
	In Traps	Window trap total		29	45	36	95	229	231	240	315	181	307	156	229	68	208	160	83	17
		Louvre trap total..		4	1	5	2	7	13	13	15	17	9	9	10	2	9	6	4	2
		Fed	Alive ..	21	20	22	33	77	75	104	90	63	56	100	65	27	28	52	28	9
			Dead ..	0	1	2	12	1	1	4	0	1	1	1	2	0	0	0	0	0
		Unfed	Alive ..	12	22	9	35	152	160	135	226	122	228	53	160	37	178	102	56	9
			Dead ..	0	3	8	17	6	8	10	14	12	31	11	12	6	11	12	3	1
BHC hut	In Hut	Fed	Alive ..	23	0	0	2	7	18	25	26	5	8	29	40	20	19	10	13	3
			Dead ..	0	2	13	5	29	12	54	33	26	20	19	16	5	4	6	0	0
		Unfed	Alive ..	3	0	0	0	0	12	1	3	0	1	2	4	0	2	0	0	0
			Dead ..	0	2	2	2	19	8	5	11	12	4	3	2	0	0	1	0	0
	In Traps	Window trap total		38	9	37	114	360	229	206	328	323	153	62	118	46	40	59	21	9
		Louvre trap total..		9	2	2	10	9	16	11	15	18	20	22	16	7	9	8	6	2
		Fed	Alive ..	30	0	0	12	18	32	38	51	30	37	35	36	28	21	20	13	7
			Dead ..	0	1	13	21	26	6	11	2	4	1	0	1	1	0	0	0	0
		Unfed	Alive ..	17	0	0	8	53	98	100	181	221	94	39	89	20	26	41	12	4
			Dead ..	0	10	26	83	272	109	68	109	86	41	10	8	4	2	6	2	0
Control hut	In Hut	Fed	Alive ..	17	14	49	77	176	172	234	342	342	173	158	192	68	64	145	81	20
			Dead ..	0	0	0	0	0	0	0	3	0	1	2	2	0	0	0	0	0
		Unfed	Alive ..	3	7	4	32	2	5	1	16	2	0	0	0	0	6	0	6	0
			Dead ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	In Traps	Window trap total		20	36	31	40	57	65	54	130	118	62	48	106	24	46	119	39	20
		Louvre trap total..		14	1	6	9	5	0	10	11	14	7	7	10	7	6	10	2	7
		Fed	Alive ..	23	20	30	26	25	24	34	72	32	28	20	39	20	28	34	20	15
			Dead ..	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
		Unfed	Alive ..	11	17	6	23	35	41	30	50	88	36	27	66	9	20	89	19	12
			Dead ..	0	0	1	0	2	0	0	19	12	5	8	10	2	3	6	2	0

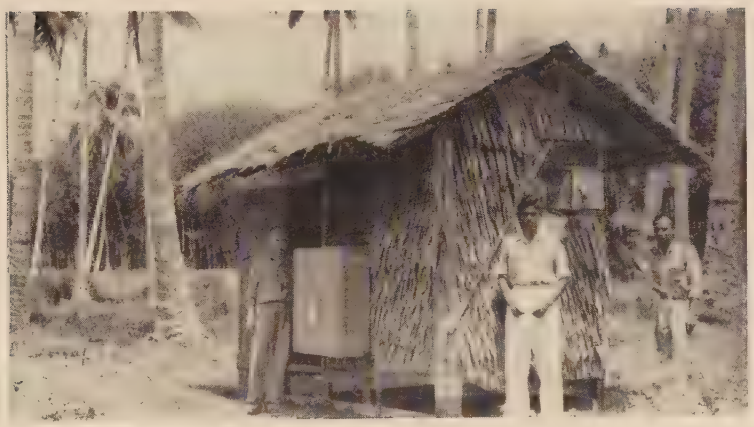

FIG. 1. General view of experimental trap hut with light and louvre traps in position.

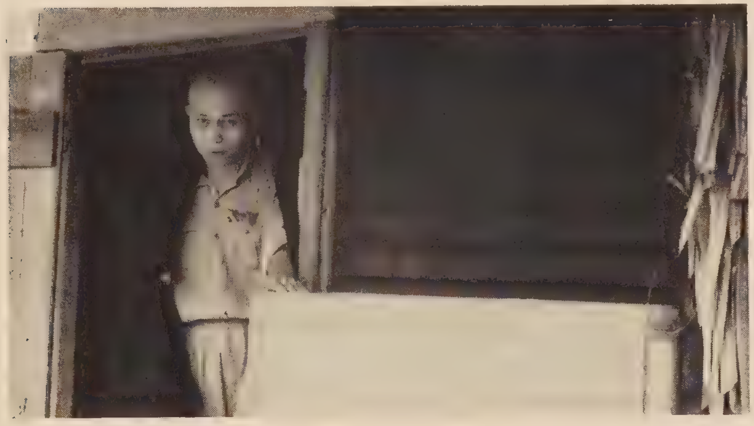

FIG. 2. Door curtains, louvres and portion of the louvre trap.

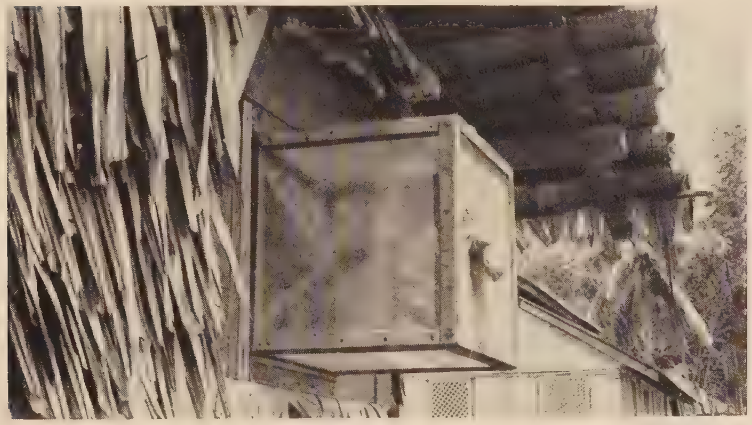

FIG. 3. Light trap fixed over window of experimental hut. Labourers' lines in background.

NOTES ON INSECTS ASSOCIATED WITH *PINUS RADIATA* IN NEW ZEALAND.

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In 1932, Clark investigated the exotic and indigenous insect relationships with *Pinus radiata* in New Zealand, recording six species in the former group and 19 in the latter. Three previously unrecorded insect associations are now added to this list.

Sirex noctilio F. is the principal insect concerned with destruction of trees in *P. radiata* forests in this country, against which the Ichneumonid parasite, *Rhyssa persuasoria* (L.) has been employed for some years by the Cawthron Institute. During the last seven years the writer has been concerned with obtaining field material of *Rhyssa* from *P. radiata* and rearing it in insectaries for distribution in New Zealand. In August and September dead trees are felled and are examined for the presence of *Sirex* and *Rhyssa* larvae. Those which contain *Rhyssa* larvae are then sawn into six foot logs and are stored in the insectaries, awaiting the emergence of *Rhyssa.* While making routine examinations of these dead *P. radiata* trees in the Belgrove plantation area in 1948, for the purpose of obtaining *Rhyssa* infested logs, on two occasions primary parasitism of *S. noctilio* by the native Oryssid wasp, *Guiglia schauinslandi* (Ashm.) was noticed. Pupae of *G. schauinslandi* were recovered from the inside of larval remains of *S. noctilio ;* in one instance this occurrence was detected only by an outline of the *G. schauinslandi* pupa inside the *Sirex* larva and was revealed on opening up the latter.

The probable native hosts of *G. schauinslandi* are the weevils, *Psepholax sulcatus* White, *P. coronatus* White and *P. barbifrons* White, as was noted by the writer in 1927. By a coincidence, *P. coronatus* has been demonstrated by Clark (1932) to have transferred its attentions additionally to *Pinus radiata,* although the writer has not yet found this relationship to be present in the Nelson province. *G. schauinslandi* must parasitise late larval stages of *S. noctilio,* when they travel to the final horizontal pupation position, close to the outside of the trunk of the tree, for they would then be within reach of the parasite's short ovipositor ; emergence of the parasite is in the October-December period, with only a few adults remaining alive by early in January, while emergence of *Sirex* does not commence in Nelson until mid January and continues until April. No young *Sirex* larvae would therefore be available for parasitism by *Guiglia.* In some instances *Rhyssa* may be hyperparasitised by *Guiglia,* for *Rhyssa* parasitises one-half to two-thirds grown larvae of *Sirex* when they are well within the tree and these larvae come close to the outside of the tree before they are finally killed and consumed by the *Rhyssa* larvae. *Rhyssa* larvae are therefore in the same vulnerable position for hyperparasitism as are *Sirex* larvae for primary parasitism. The significance of *Guiglia* cannot yet be assessed, but the percentage of parasitism must be low, for only a few dozen individuals appear in the insectaries every year, compared with some thousands of *Rhyssa* and even greater numbers of *Sirex.*

The other two occurrences on *P. radiata* are both of indigenous beetles, *Stenopotes pallidus* Pasc. (Cerambycid) and *Anthribus sharpi* Broun (Anthribid), which spend their larval instars in and under the bark of dead trees at the same state of desiccation as the *Sirex-Rhyssa* relationship.

The records of an Oryssid parasitising *S. noctilio* and of the two additional native beetles in association with *P. radiata* are additional interesting examples of the manner in which certain members of the New Zealand insect fauna are adapting themselves to the changing biological relationships in areas in which the native forest cover has been greatly reduced by milling or fire. This restricts indigenous insects to the remaining native forest, thus limiting their distribution, which is increased only when a new association is formed with exotic trees or insects.

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THE BIOLOGICAL CONTROL OF *GONIPTERUS SCUTELLATUS* GYLL. (COL. CURCULIONIDAE) IN MAURITIUS.

By

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Historical Account.

A considerable degree of control of the Eucalyptus Snout Beetle, *Gonipterus scutellatus* Gyll., has been achieved in Africa by means of the egg parasite, *Anaphoidea nitens* Gir. (Mymaridae). The host and parasite are both of Australian origin (Mally, 1924). Early in the present century, *Gonipterus* spread to New Zealand and also to South Africa (*l.c.*), being first discovered in the latter country in 1916. It eventually became a major pest of several species of *Eucalyptus* in South Africa, and the difficulty of controlling it by insecticides led to a search for parasites in Australia. This resulted in the discovery of *A. nitens*, which was introduced into South Africa in 1926 (Tooke, 1942), and has given excellent control of *Gonipterus* in most areas. The weevil, however, continued to spread ; in 1937 both host and parasite were present in the southern part of Nyasaland (Smee, 1937) ; in 1940 both were recorded in Southern Rhodesia (anonymous, 1940) ; in 1944 *Gonipterus* was observed at Kericho, Kenya, and subsequently in several other areas and also in Eastern Uganda. The insect had not spread unaided to these areas, for its parasite was absent. *Anaphoidea* was imported subsequently and has now become firmly established (Kevan, 1946).

Gonipterus adults were first collected in Mauritius in 1940, but no importance was attached to their identification until an infestation of *Eucalyptus* trees was observed in November, 1943 (Moutia & Vinson, 1945).

More recently *Gonipterus* has appeared in Madagascar, where measures have been taken to control it by the introduction of *A. nitens* (Williams, 1949).

Gonipterus in Mauritius.

Serious attention was first drawn to *G. scutellatus* in February 1944, when extensive attacks by an unknown insect were reported from *Eucalyptus* plantations. As soon as it had been identified an island survey was carried out, and it was found that the weevil was present in most districts. Of the three species of *Eucalyptus* extensively cultivated, *E. robusta*, *E. tereticornis*, and *E. kirtoniana*, the former was liable to the most serious attacks. A search was made for natural enemies of the insect during this survey, in the course of which more than 2,000 egg capsules were collected. *A. nitens* proved to be absent, but adults were sometimes found attacked by an entomogenous fungus, probably of the genus *Hirsutella* (Moutia & Vinson, 1945).

The threat to *Eucalyptus* trees had obviously been developing for several years, for as mentioned above, some adults had been captured, but not identified, in 1940. The entry of *Gonipterus* into the island must be presumed to have occurred two or three years previous to this date.

In view of the considerable degree of control over the pest achieved by *A. nitens* on the African continent, the importation of this parasite was initiated.

The Introduction and Establishment of *Anaphoidea*.

Introduction.

Owing to transport difficulties resulting from the war, two air consignments of about 10,000 parasitised *Gonipterus* egg capsules, sent through the courtesy of Dr. Naudé, Chief Entomologist of the Union of South Africa, in 1946, were unsuccessful. Delays in transit resulted in the emergence of the parasites en route, and those still alive on arrival died within two days without ovipositing. A further importation was arranged from East Africa, and 780 egg capsules collected and conveyed to Mauritius by Mr. P. Regnard, produced 165 *Anaphoidea*. With this material, laboratory breeding enabled 12,500 adults to be released in 36 districts in colonies of 150 to 800, with an average of 347 per colony. These liberations took place beween May and September 1946.

The breeding of *Anaphoidea* was carried out in glass cylinders 5 ins. by 1 in. closed at both ends with a perforated cork covered with muslin. Pieces of *Eucalyptus* leaves with *Gonipterus* egg capsules were placed in the cylinders with a few *Anaphoidea* and left for one or two days depending upon the number of capsules used. With larger containers, oviposition was reduced. Food was supplied by smearing honey on one side of a slip of paper riddled with pin-holes, the slip then being stuck to the wall of the cylinder. This method enabled the parasites to feed through the pin-holes without becoming stuck in the honey. No moisture was supplied, as that given off by the pieces of *Eucalyptus* leaves was sufficient.

Establishment.

Examination of *Gonipterus* egg capsules from 19 areas in December 1946 showed that the parasite had already become firmly established and was rapidly dispersing, recoveries having been made several miles from the points of release.

In February–June 1947, 54 samples of egg capsules from various Crown Lands and private properties all proved to be parasitised with a mean cluster parasitism of 27·7 per cent., and a maximum of 76 per cent. A reduction in the intensity of *Gonipterus* attacks was already becoming evident in several areas where damage had been consistently high (Moutia, 1948).

Two island surveys were carried out during 1948, one in July–August and the other in November–December, when egg capsules were collected from 21 localities in each case. The results of these surveys are given in Table I. A rough idea of the abundance of *Gonipterus* in each locality at the time of the survey is given by the number of capsules collected, for no attempt was made to obtain numerous capsules if they were scarce. The capsules were kept separately in small tubes with the minimum of leaf. The percentage of eggs parasitised was obtained for each locality by counting the *Gonipterus* larvae and *Anaphoidea* adults emerging from the capsules. When emergence had ceased, the capsules were examined for parasites which had failed to emerge, and any so found were taken into account—these amounted to 18·8 per cent. of the total number of *Anaphoidea*. *Gonipterus* egg mortality from causes other than *Anaphoidea* was negligible, and was accordingly ignored. The figures for the average number of *Anaphoidea* per cluster were derived from the parasitised clusters of each locality only.

The means of egg parasitism for the two surveys are 29·3 per cent. and 39·5 per cent. respectively. The individual figures for each locality are, however, of greater significance as they vary, excluding those localities where the parasite is absent, from 1·1 per cent. to 99·4 per cent. in the July–August survey, and from 7·6 per cent. to 86·6 per cent. in the November– December survey, and in several localities there is a wide difference between the percentage egg parasitism in each survey. These variations are discussed later.

TABLE I.

Locality	July—August, 1948				November—December, 1948			
	No. clusters collected	% clusters parasitised	% eggs parasitised	Average No. *Anaphoidea* per parasitised cluster	No. clusters collected	% clusters parasitised	% eggs parasitised	Average No. *Anaphoidea* per cluster
Solitude	82	23·1	21·2	6·1	19	42·1	23·0	5·5
Abercrombie	9	11·1	2·5	1·0	25	20·0	11·9	4·4
Schoenfeld	28	25·0	14·2	4·3	36	20·6	8·9	3·1
Piton	62	1·6	1·1	5·0	19	0	0	0
St. Antoine	89	17·9	15·9	7·0	29	82·7	68·3	8·4
Nouvelle Découverte (a) ..	23	52·1	40·9	5·2	59	94·9	81·5	9·1
,, ,, (b) ..	7	40·0	56·1	9·2	—	—	—	—
,, ,, (c) ..	37	73·3	70·1	9·0	—	—	—	—
Brisée Verdière	23	91·3	79·1	5·8	21	95·2	59·0	5·9
Cluny	64	100·0	99·4	6·1	15	93·3	38·0	6·4
Floréal	84	11·9	8·3	5·4	26	86·9	66·9	6·7
St. Julien	56	10·7	8·5	6·8	10	50·0	36·9	7·6
Tamarin	11	0	0	—	0	0	0	—
Casela	10	50·0	43·7	6·2	16	81·2	64·7	5·8
La Ferme	10	0	0	0	18	33·3	7·6	2·3
Reduit	59	76·2	70·1	6·9	29	93·1	61·8	7·8
Chamouny	45	6·6	5·5	8·0	12	58·3	49·4	6·1
Baie du Cap	1	0	0	0	5	0	0	0
Beau Vallon	47	17·4	8·4	4·2	25	12·0	8·8	7·3
Mouna	33	21·2	16·1	6·8	29	41·4	19·1	5·0
Beau Bassin	—	—	—	—	30	80·0	55·2	5·2
Quartier Militaire	—	—	—	—	29	96·5	86·6	9·6
Mare aux Vacoas	115	71·3	54·3	6·0	39	97·4	82·3	7·3
Totals of clusters collected ..	895				491			
Means		33·3	29·3	6·0		56·1	39·5	5·4

Notes on the Biology of *A. nitens.*

The biology of *Anaphoidea* in South Africa has previously been described by Mossop (1929). The reader is referred to this paper, and the present notes are merely intended to furnish additional information on the bionomics of this important parasite under Mauritian conditions.

The female is distinguished by the enlarged terminal segment of the antenna. In the laboratory, mating occurs very soon after emergence and oviposition commences on the same day. In common with most Mymarids, *A. nitens* is solitary, only one parasite developing within a host egg. Pupation occurs within the chorion of the host egg, the adult emerging from the egg mass, or capsule, by chewing a small round hole. Several parasites may escape through one hole, while others may escape through the hole made beneath the capsule by hosts which have escaped parasitism.

Oviposition and developmental period.

In order to determine the fecundity of the female parasite, ten copulating pairs were selected from freshly emerged individuals and each pair separated in tubes 3 ins. by ¾ in. plugged with slightly damp cottonwool which had been lightly smeared with honey. Batches of ten freshly laid *Gonipterus* egg clusters were introduced into each tube and left for 24 hours. The clusters were then removed and replaced by fresh batches which were also left in contact with the parasites for 24 hours. A third series of egg clusters was left in the tubes for 48 hours, and a fourth until the death of the female. Each batch of ten clusters so treated was kept separately in tubes and examined daily for emerging parasites. The data so obtained is given in Table II. The parasites emerged from the capsules between 12 and 15 days after oviposition.

TABLE II.

Copulating Pair	Number of eggs laid during				Total eggs laid	Life of ♀ in days
	1st day	2nd day	3rd and 4th days	5th day +		
1	19	8	5	0	32	8
2	15	7	4	1	27	6
3	12	7	3	1	23	9
4	14	8	3	0	25	6
5	11	10	6	1	28	7
6	11	10	5	0	26	6
7	13	9	5	1	28	7
8	14	10	5	0	29	4
9	13	9	5	0	27	7
10	13	7	3	0	23	3
Means ..	13·5	8·5	4·4	0·4	26·8	6·3

It will be seen that a female lays about 27 eggs and that, with an excess of host material, oviposition ceases three or four days after mating, about half the total number of eggs being deposited within the first 24 hours.

Age of host eggs.

Parasitism of egg clusters of six or more days old could not be obtained in the laboratory, while the degree of parasitism in clusters four days old was lower than usual, and that of five days old clusters negligible.

Sex ratio.

Under field conditions the sex ratio, as determined from over 700 specimens emerging from parasitised capsules, approximates two males to three females. When parthenogenesis occurs the progeny are all males. The preponderance of females in the field shows that parthenogenesis is unusual and perhaps abnormal ; in a laboratory experiment in which twenty unfertilised females were offered fresh egg capsules for oviposition, only one produced progeny.

Notes on the Biology of *G. scutellatus.*

The biology of *G. scutellatus* in South Africa has been described by Mally (1924), while Moutia & Vinson (1945) have given an account of its life-cycle in Mauritius. The two latter workers stated that the number of eggs per capsule varies between eight and twelve, and breeding continues throughout the year, there being at least four generations. Table III giving the duration of the life-cycle at different periods of the year is adapted from the table given in their paper.

TABLE III.

	February–March	April–June	July–August
Egg	6–7 days	7–9 days	9–10 days
1st larval instar	3–4 ,,	4–5 ,,	5–6 ,,
2nd ,, ,, ...	3–4 ,,	4–6 ,,	6–7 ,,
3rd ,, ,, ...	3–4 ,,	4–6 ,,	5–8 ,,
4th ,, ,, ...	5–6 ,,	6–8 ,,	7–10 ,,
Pupa	29–31 ,,	31–35 ,,	32–38 ,,
Total	49–56 ,,	56–69 ,,	64–79 ,,
Mean Temp.	27·0°C.	23·6°C.	21·3°C.
Max. ,, ...	31·6°C.	28·9°C.	25·5°C.
Min. ,, ...	24·4°C.	20·0°C.	17·8°C.

The reproductive capacity of the weevil was also determined. Freshly emerged adults were placed in jars containing *Eucalyptus* twigs in water. Mating occurred 4–9 days later, and ten copulating pairs were isolated and accommodated similarly. The first egg clusters were laid 13 to 21 days after mating ; the total number of clusters laid by each female was between 21 and 33. Taking an average of ten eggs per cluster a female may thus be said to lay a total of about 270 eggs. Egg laying continued throughout the life of the females, which averaged 91 days, during which time several matings occurred.

Discussion.

The introduction of *A. nitens* and its extremely rapid establishment and dissemination over the island has proved a most effective check to *G. scutellatus*. Whilst, just previous to the release of the parasite, the ravages of the weevil had reached the stage when further plantation of *Eucalyptus* was regarded with concern, *Gonipterus* is now reduced to a pest whose attacks are sporadic and localised. Observations during 1948 and 1949 have shown that such outbreaks pass before damage becomes extensive. This is attributed wholely to *Anaphoidea*, for after these attacks egg parasitism is always high.

Previous utilisation of *Anaphoidea* has been restricted to the African continent, where results, although on the whole excellent, have been erratic owing to the effects of the very diverse climatic conditions in different areas upon the host which, for instance, hibernates in the adult stage for many months in the highveld of South Africa (Tooke, 1942). Such wide climatic differences do not exist in oceanic Mauritius and control is uniform over the island. The same author, however, reports very pronounced differences in the degree of control within tracts where climatic conditions are uniform. This is comparable with the localised outbreaks in Mauritius. It is difficult to explain such local variations in the parasite-host ratio when the parasite shows such a marked ability to disperse rapidly. The parasite surveys of Table I show very great differences of percentage egg parasitism between different localities and also in the same localities at different times of the year. The dynamic nature of these survey figures is to be emphasised ; the cause of the rapidly changing host-parasite ratio undoubtedly being the wide disparity of the respective reproductive rates. More, however, is needed to explain the localised nature of those fluctuations.

Mauritius is frequently subjected to cyclonic disturbances, and it was thought earlier that the population of *Anaphoidea* might be drastically reduced from time to time by the high winds that accompany these disturbances, as in the case of the introduced Scoliid parasites of the Sugar-cane White Grub, *Clemora smithi* (Arrow). Violent gales have, however, been experienced without any detrimental effect, and it seems plausible to assume that the protection offered to the immature stages by the host capsule, together with the short life-cycle, soon counterbalances the loss of a considerable part of the adult population.

Summary.

A brief historical account is given of the spread of the Eucalyptus Snout Beetle, *Gonipterus scutellatus* Gyll., fron Australia, its country of origin, and of the utilisation of its natural parasite *Anaphoidea nitens* Gir. for its control on the African mainland.

The discovery of *G. scutellatus* in Mauritius is described. Its entry was effected between 1935–40, and by 1944 it was threatening *Eucalyptus* plantations over the whole island.

A. nitens was introduced in 1946 from East Africa. Establishment was extremely rapid and two years later *G. scutellatus* was effectively controlled.

Notes are given on the bionomics of both host and parasite under Mauritian conditions.

Ecological data are briefly discussed.

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THE EFFECTS OF HIGH TEMPERATURE ON THE COMMON CLOTHES MOTH, *TINEOLA BISSELLIELLA* (HUMM.).

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(Plates II and III.)

The heat treatments required to kill *Tineola bisselliella* (Humm.) have been determined to some extent, but mainly at temperatures over 43°C. Titschack (1925) investigated the influence of temperature on growth rate and length of life, using temperatures up to 30°C. He also experimented on the heat resistance of all stages of the insect's life-history using temperatures of 44°C. to 73°C. with very short exposures of 12 minutes or less. Back (1923) recommends exposures of 31 minutes or less at temperatures ranging from 43°C. to 53°C. for disinfesting articles, and Wallace & others (1926) recommend 55°C. for at least 24 hours for practical application. Mellanby (1934) used temperatures of 30°C. and 35°C. in his experiments on metabolism but with exposures that did not cause death.

Wallace's specifications obviously include a generous allowance for penetration of heat through materials surrounding the insects, but no specific data for this are given. This aspect of heat treatment is clearly important in practice and is now being investigated, but in the present paper this factor is excluded and the heat treatments specified apply to unprotected insects.

From the point of view of practical control, knowledge of the exposure periods that would be lethal at temperatures between 30°C. and 43°C. would be valuable, as these conditions should be attainable in private houses, etc., without too much difficulty.

This range of temperatures was therefore selected for the present investigation, which was carried out in order to determine :—

(a) The lowest temperatures, applied for specified periods, that are lethal to all stages of the insect.

(b) The percentage mortality induced by different exposures at different temperatures :—

    (1) during, or immediately after the exposure ;

    (2) after a lapse of time (delayed effects).

(c) The highest temperature which allows complete development of all stages.

(d) The conditions necessary to prevent the laying of fertile eggs.

Methods of Culturing.

The first experiments were carried out on insects taken from stock cultures in the insectaries. These cultures are provided with pieces of pure wool flannel and with a mixture of 18 parts of fish-meal to 1 of yeast, and are maintained at 24°C. and 70 per cent. relative humidity.

The larvae of the clothes moth thrived upon this diet, but it was difficult to select individual insects to use in experiments, as they became hidden in the food material. It was also difficult to select stages of known age from the cultures, as these were set up with adult moths and therefore eggs were laid over long periods.

A new method of rearing stocks was therefore adopted, based on that used at the laboratories of the Wool Industries Research Association at Leeds (Fraenkel & Blewett, 1946 ; Barritt & Hartley, 1948). Strips of flannel approximately 4 ins. wide were passed through a 10 per cent. suspension of ten parts by weight of brewers' yeast, and one part of cholesterol in distilled water. These strips were then put through a small domestic wringer so that any surplus liquid was squeezed out and the flannel remained impregnated with yeast and cholesterol (Plate II, fig. 1). When dry, the strips were folded as shown in Plate II, fig. 2, and placed in a 7-lb. glass jar so that they could be opened out easily and the insects picked off.

On this diet *T. bisselliella* completes its development in about 6 weeks at 25°C. and 70 per cent. relative humidity. A new culture was set up twice a week with approximately 1,000 eggs. The eggs were obtained by placing about 100 adults in a cylindrical tin which had a 14 mesh per inch metal gauze base, and standing the tin on a piece of wool gabardine. The moths laid through the gauze, and when the tin was lifted away, the eggs could be brushed off the gabardine (Plate III, figs. 1 and 2). When setting up cultures, eggs up to 3 days old were used.

Experimental Method.

All the experiments were carried out either in incubators or in a water bath. The water bath consisted of a glass battery jar 17 ins. high by 14 ins. by 10 ins. containing rainwater and a small amount of mercuric cyanide. It was fitted with a 500-watt immersion heater and a stirrer ; a bimetallic thermostat having a range of 25°–45°C. and a sensitivity of ± 0·5°C. provided the temperature control. A copper gauze basket with six compartments was suspended in the water so that tubes, 6 in. by $1\frac{1}{2}$ in., placed in it were covered by water almost to the top (Plate III, fig. 5). Five of these large tubes contained a small 2 in. by 1 in. tube holding the insects and the sixth contained a smaller tube with a thermometer passing into it. All the tubes were fitted with rubber bungs.

The required humidity was maintained in the incubators by conducting all experiments in desiccators containing an appropriate solution of caustic potash. When using the water bath, the correct humidity was obtained by conditioning the inner tubes and their contents in desiccators and then maintained by corking tightly with rubber bungs. Preliminary tests showed that the required humidity was maintained inside the tubes for at least 24 hours.

In the experiments in incubators, the insects were placed in 2 in. by 1 in. tubes fitted with corks through which a hole had been bored and then covered with muslin. Eggs were placed on circles of dark blue wool gabardine which fitted the base of the tubes and larvae, pupae and adults in tubes lined with food-impregnated flannel.

All material after exposure was transferred to a constant temperature room at 25°C. and 70 per cent. relative humidity and examined at intervals.

Results.

(i) *Eggs.*

Eggs up to 24 hours old were transferred from the gabardine of the laying cage to the tubes with a small paint brush, all damaged eggs being discarded. Experiments involving exposures of 24 hours or less were performed in the water bath, all others being carried out in the incubators.

Eggs that had been exposed for a prolonged indefinite period were examined regularly until they had become collapsed and shrivelled and would obviously never hatch.

Temperatures between 30°C. and 41°C. and exposure periods from four hours upwards were used. The effect of different relative humidities on the lower temperatures was insignificant and was not tested at the higher temperatures.

The average (control) mortality of eggs is approximately 25 per cent. (J. F. Basden, unpublished report).

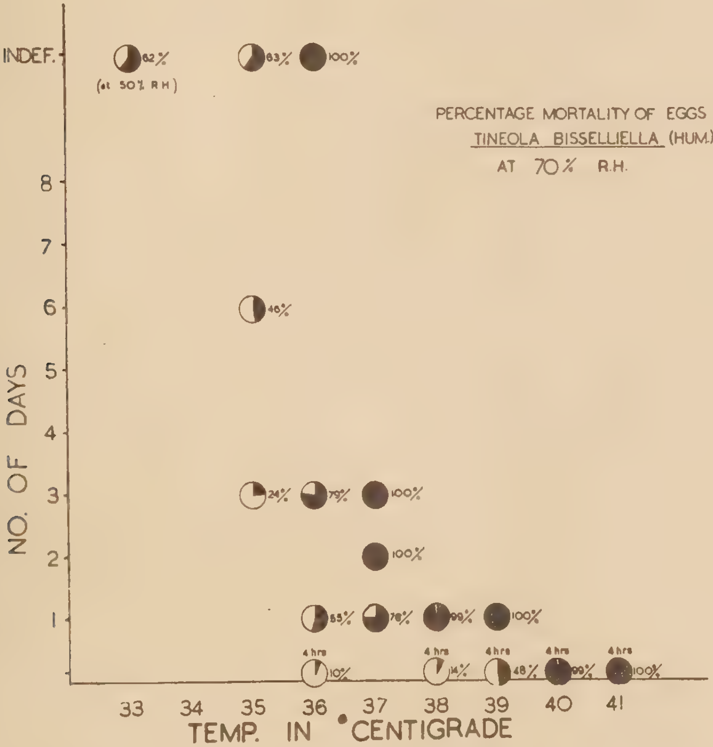

Fig. 1.—Percentage mortality of eggs of *Tineola bisselliella* after exposures at various time-temperature combinations. When exposure periods are less than 1 day, the time is inserted above the symbols.

The results of the experiments are illustrated in Table I and fig. 1. It will be noted that mortality at 30°C. was not significantly greater than normal, but at temperatures of 35°C. and over it varied with the exposure. At 70 per cent. relative humidity 100 per cent. mortality was obtained after prolonged exposure at 36°C. two days at 37°C. ; one day at 39°C. and four hours at 41°C.

The incubation period of eggs at temperatures over 34°C. varied. The longest was 12 days, after an exposure to 35°C. and 70 per cent. R.H. for three days. The shortest was four days. occurring at various exposures, but the majority of eggs hatched in five to nine days.

TABLE I.

Mortality of eggs of *T. bisselliella.*

Temperature	Relative humidity	Length of exposure	Number of eggs	Number not hatched	Percentage mortality
20°C.	70%				24·87%
30°C.	30%	∞	100	27	27%
	50%	,,	200	57	28·5%
	70%	,,	100	32	32%
	90%	,,	200	35	17·5%
33°C.	30%		150	88	59%
	50%	,,	100	62	62%
	90%	,,	100	63	63%
35°C.	70%	∞	100	63	63%
		6 days	50	23	46%
		3 ,,	50	12	24%
36°C.	70%	∞	50	50	100%
		3 days	100	79	79%
		1 day	90	50	55%
		4 hours	50	5	10%
37°C.	70%	3 days	50	50	100%
		2 ,,	50	50	100%
		1 day	100	76	76%
38°C.	70%	1 day	100	99	99%
		4 hours	50	7	14%
39°C.	70%	1 day	50	50	100%
		4 hours	150	72	48%
40°C.	70%	4 hours	100	99	99%
41°C.	70%	4 hours	100	100	100%

∞ = exposure prolonged indefinitely.

(ii) *Larvae.*

Experiments were carried out on larvae from cultures about four weeks old. At this age they were considered to be fully grown. In some preliminary experiments, when insectary stocks were used, larvae were chosen by their size, as it was impossible to calculate age.

Larvae were picked out of the cultures and transferred to tubes by forceps. The 2 in. by 1 in. tubes were lined with impregnated flannel similar to that used in the cultures. The food material was conditioned to 70 per cent. relative humidity and, in the case of short exposures using the water bath, the 2 in. by 1 in. tubes and the food material were first brought up to the experimental temperature and the larvae were quickly dropped in. With exposures of 24 hours or longer, which were carried out in incubators, the tubes were cool at the commencement of the exposure.

Mortality in the control was nil ; all fully grown larvae that were uninjured developed into pupae.

In all experiments, a larva was considered dead when it did not respond to touch in any way. The results of these experiments are set out in Table II and fig. 2.

TABLE II.

Mortality of larvae of *Tineola bisselliella.*

Temperature	Humidity	Length of exposure	Number of larvae exposed	Mortality on day after end of exposure	Per-centage Total	Mortality 10 days after end of exposure	Per-centage	Ultimate Total Mortality	Per-centage	Maximum survival time* (days)
33°C.	70%	∞	25	0	0	8	32%	22	88%	303
35°C.	50%	1 day	15	0	0	4	27%	4	27%	—
		6 hrs.	15	0	0	3	20%	3	20%	—
	70%	∞	145	0	0	41	28%	145	100%	106
		8 days	25	0	0	0	0	4	16%	53
		4 ,,	25	0	0	2	8%	6	24%	36
		1 day	25	0	0	2	8%	6	24%	13
36°C.	70%	4 days	25	2	8%	10	40%	20	80%	16
		3 ,,	25	2	8%	8	32%	16	64%	13
37°C.	70%	7 days	25	25	100%	25	100%	25	100%	7
		6 ,,	25	22	88%	25	100%	25	100%	10
		3 ,,	25	18	72%	21	84%	22	88%	28
		1 ,,	50	14	28%	37	74%	41	82%	13
		4 hrs.	25	0	0	4	16%	6	24%	12
		3 ,,	25	0	0	0	0	6	24%	30
38°C.	70%	1 day	25	25	100%	25	100%	25	100%	2
		18 hrs.	25	25	100%	25	100%	25	100%	2
		4 ,,	25	2	8%	8	32%	15	60%	14
39°C.	70%	3 hrs.	25	0	0	25	100%	25	100%	3
		2 ,,	25	6	24%	24	96%	25	100%	57
		1½ ,,	50	1	2%	6	12%	32	64%	21
		1 hr.	25	0	0	3	12%	7	28%	18
40°C.	70%	3 hrs.	25	25	100%	25	100%	25	100%	1
		2 ,,	25	0	0	25	100%	25	100%	1
		1 hr.	50	0	0	34	68%	46	92%	62
41°C.	70%	3 hrs.	50	50	100%	50	100%	50	100%	1

∞ = exposure prolonged indefinitely. * From commencement of exposure.

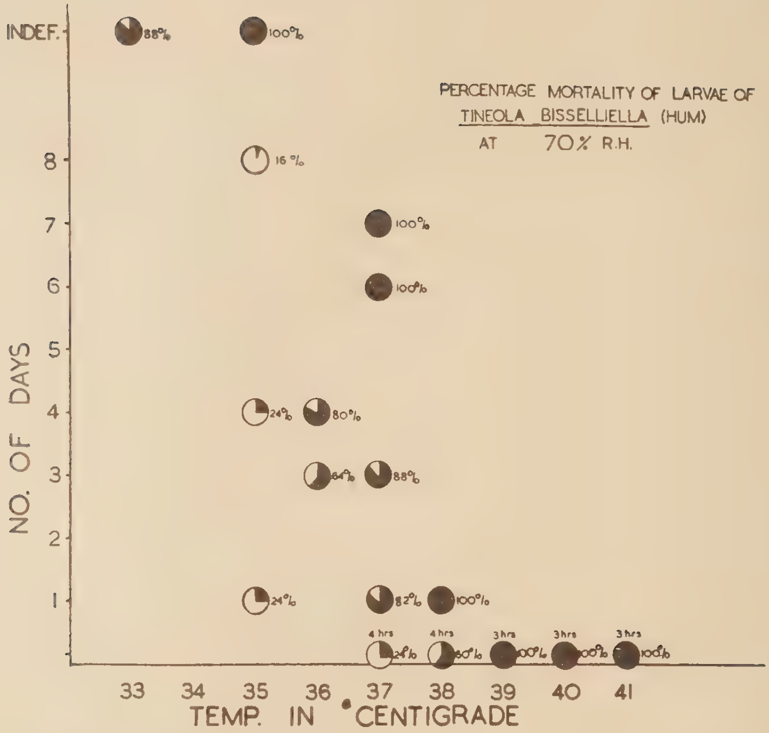

Fig. 2.—Percentage mortality of larvae of *T. bisselliella* after exposures at various time-temperature combinations. When exposure periods are less than 1 day, the time is inserted above the symbols. The results of some exposures of 3 hrs. or less are not indicated in this diagram.

It will be noted that, as with the eggs, mortality varied with the length of exposure, but there was frequently a delay in lethal effect, which mainly varied inversely with the time of exposure. With 70 per cent. R.H. prolonged exposure at 33°C. allowed only 12 per cent. of larvae to develop into pupae, whilst at 35°C., although such exposure allowed larvae to remain active and to feed for a considerable time, 100 per cent. mortality finally occurred. With the following treatments at 70 per cent. R.H., 100 per cent. mortality was also obtained: six days at 37°C.: 18 hours at 38°C.; two hours at 39°C. In some cases mortality was not complete until some days after the end of these treatments, but 100 per cent. kill occurred during or immediately after seven days at 37°C., 18 hours at 38°C. and three hours at 40°C. Normally, at temperatures of 36°C. and over, all surviving larvae completed their development and there were only two cases of larvae which pupated and these died as pupae. These occurred after exposure of three hours at 37°C. and one hour at 40°C.

A few newly hatched larvae were exposed to 30°C. only. Six hours at 35°C. caused the death of two out of the 15 exposed, and after one day's exposure only two out of 25 became adults. After two days' exposure 24 out of 25 died, and the remaining one completed its development.

(iii) *Pupae.*

Pupae were placed in 2 in. by 1 in. tubes lined with flannel, in the same way as the larvae.

At first an attempt was made to collect pre-pupae and to keep them until they became pupae, thus using pupae all of the same age. But as the pre-pupal stage lasts only three days or less and is difficult to find, this practice was abandoned and pupae of the lightest colour were selected, as pupae become darker with increasing age (Griswold, 1944). Occasionally, during long exposures, adults emerged before the end of the treatment and, in calculating results, these were counted as having emerged immediately after the treatment. It is impossible to tell precisely when a pupa is dead, so in all cases they were kept under observation until the abdomen began to shrivel. The results are shown in Table III and fig. 3.

TABLE III.

Mortality of pupae of *T. bisselliella*. (R.H. 70%.)

Temperature	Length of exposure	Number of pupae exposed	Total mortality	Percentage mortality
20°C.	∞	20	0	0
33°C.	∞	25	9	36%
35°C.	∞	25	25	100%
	6 days	40	23	57%
	4 ,,	50	18	36%
	3 ,,	10	1	10%
	2 ,,	20	4	20%
36°C.	3 ,,	25	9	36%
	1 ,,	25	3	12%
37°C.	2 ,,	25	14	56%
	1 ,,	25	15	60%
38°C.	1 ,,	50	50	100%
	4 hours	25	23	92%
39°C.	3 hours	25	21	88%
	1½ ,,	25	4	16%
40°C.	6 hours	25	25	100%
	3 ,,	25	25	100%

∞ = exposure prolonged indefinitely.

Of 20 pupae kept as a control at 20°C. and 70 per cent. relative humidity, all emerged ; this is in agreement with Griswold (1944) who found that very few pupae die under insectary conditions.

It should be noted that of 25 pupae exposed indefinitely to 33°C. and 70 per cent. R.H., 16 became adults ; one within four days, 11 within six days and four within 15 days, but no pupae developed into adults when exposed indefinitely to 35°C. at R.H. of 30 per cent., 50 per cent., 70 per cent. and 90 per cent. (the result shown in Table III is for 70 per cent. R.H. only). Mortality of 100 per cent. was also obtained with exposures of one day at 38°C. and three hours at 40°C. When pre-pupae were kept at 35°C. and 30 per cent. and 90 per cent. relative humidity, about 50 per cent. died without becoming pupae.

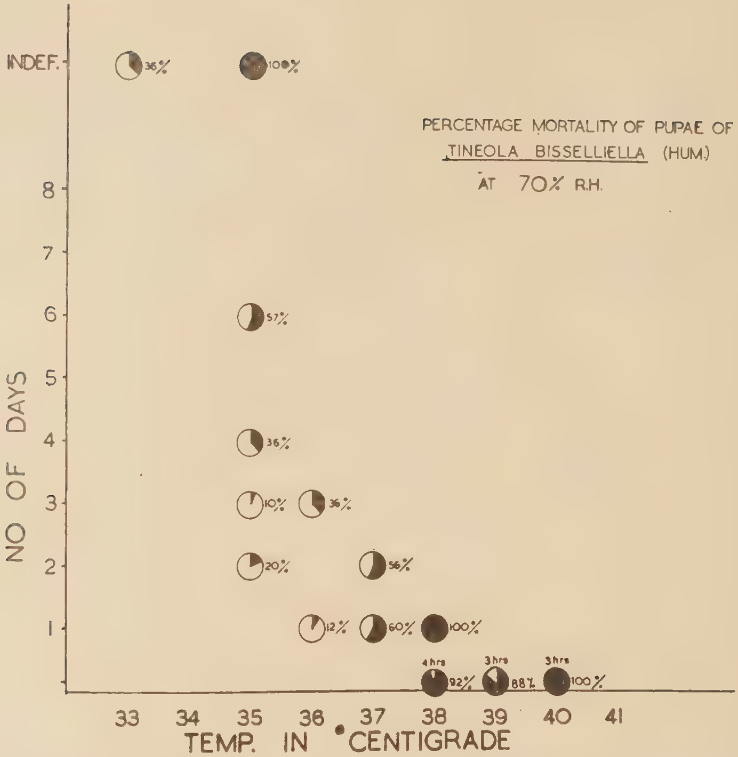

Fig. 3.—Percentage mortality of pupae of *T. bisselliella* after exposures at various time-temperature combinations. When exposure periods are less than 1 day, the time is inserted above the symbols.

(iv) *Adults.*

Young adults were obtained by clearing a culture of all adults, leaving it for not more than 48 hours, and then collecting all the newly emerged moths. These were placed in 2 in. by 1 in. tubes, five in a tube, and exposed to various temperatures in the usual way.

The mortality of the adult is only effective, from the point of view of control, if it occurs soon after emergence. The length of life varies greatly, and 75 adults kept as controls at 25°C. and 70 per cent. R.H. lived for times varying from 3–19 days. According to Titschack (1922), males may live from 4–56 days and females from 6–51, so that it is difficult to assess the mortality actually caused by heat treatment. In these experiments, the mortality was calculated one day and five days after the end of the exposure.

Adults were considered dead if there was no response to pressure on the abdomen by a small paint brush. They were not examined immediately after the end of the exposure because it was found that in some cases adults appeared dead after treatment, but revived within 24 hours.

The results of the experiments are set out in Table IV and fig. 4.

TABLE IV.

Mortality of adults of *T. bisselliella*. (R.H. 70%.)

Temperature	Length of exposure	Number of adults exposed	Mortality on day after end of exposure	Percentage	Total mortality 5 days after end of exposure	Percentage
35°C.	∞	20	0		0	
	6 days	25	25	100%	25	100%
	5 ,,	10	0	0	10	100%
	4 ,,	30	24	80%	29	97%
	3 ,,	30	5	17%	18	60%
	2 ,,	10	2	20%	2	20%
	1 ,,	25	0	0	4	16%
36°C.	4 ,,	25	25	100%	25	100%
	3 ,,	25	25	100%	25	100%
	2 ,,	25	21	84%	22	88%
	1 ,,	50	2	4%	18	36%
37°C.	3 ,,	25	25	100%	25	100%
	1 ,,	75	49	65%	58	77%
	4 hrs.	25	0	0	5	20%
38°C.	1 day	25	25	100%	25	100%
	4 hrs.	25	1	4%	7	28%
39°C.	1 day	25	25	100%	25	100%
	4 hrs.	25	23	92%	25	100%
40°C.	4 hrs.	25	25	100%	25	100%

∞ = exposure prolonged indefinitely.

It was found that all moths were dead after exposure for six days at 35°C., three days at 36°C. and 37°C., one day at 38°C. and 39°C., and four hours at 40°C. Within five days after the conclusion of the treatment, 100 per cent. mortality had resulted from five days at 35°C. and four hours at 39°C.

When less severe treatments are imposed, the survival times are subject to considerable individual variation. For example, after two days at 36°C., 21 out of 25 adults died immediately, but one lived for 16 days and one for 21 days. After one day at 37°C. 16 out of 25 died immediately, but five lived 12 days, and one lived 15 days. But after one day at 36°C., 25 moths died at an average rate of about one per day for 22 days, and the same occurred after an exposure of four hours at 38°C.

Although moths are not killed at some temperatures they may be made sterile. A few experiments were made at 35°C. Moths of one sex were exposed to this temperature for a certain length of time and then paired with a newly emerged individual of the opposite sex, and any eggs laid were kept under observation. When female moths were exposed for two days and then mated, the eggs laid after fertilisation were viable, but when male moths were exposed for the same length of time and mated with virgin females, the eggs laid by the females did not hatch. Females exposed for three days at 35°C. also laid viable eggs when mated.

Discussion.

It is apparent from the experiments described that all stages of *Tineola bisselliella* can be destroyed by comparatively low temperatures. The minimum exposure that would be completely effective is four hours at 41°C. although, except for the eggs,

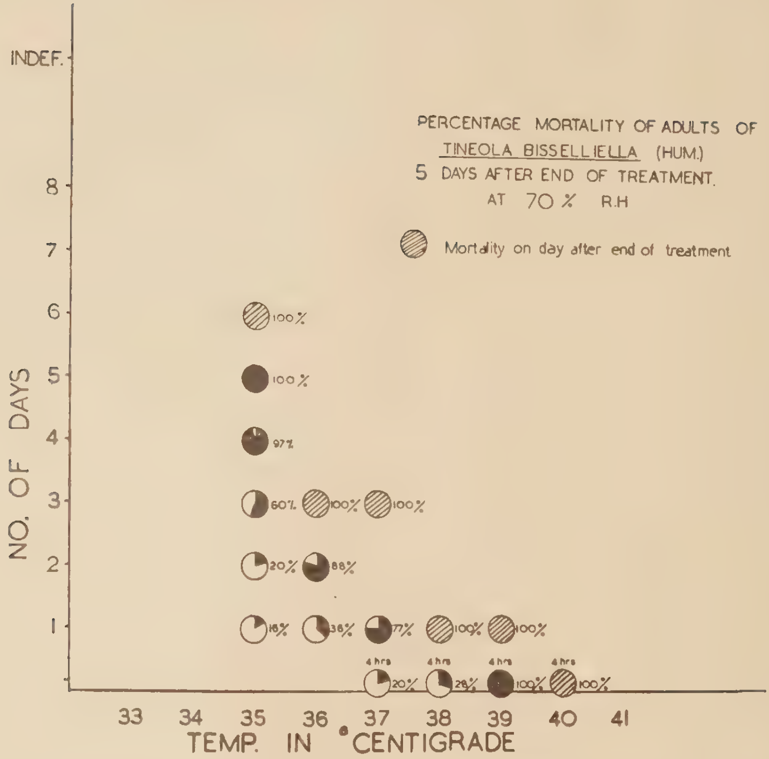

Fig. 4.—Percentage mortality of adults of *T. bisselliella* after exposures at various time-temperature combinations. When exposure periods are less than 1 day, the time is inserted above the symbols.

which are the most resistant stage at temperatures of 38°C. and over, 40°C. for this time would be sufficient. One day at 38°C. destroyed all stages except the eggs, one per cent. of which survived this exposure.

At temperatures below 38°C., the larvae and pupae can survive longer exposures than the eggs. For example, two days at 37°C. is completely lethal to eggs (fig. 1), but kills only 56 per cent. of the pupae (fig. 3) and between 82 per cent. and 88 per cent. of the larvae (fig. 2).

Table V indicates the length of exposures required to produce 100 per cent. mortality at temperatures from 35°C. to 41°C. The times shown are those determined by experiment and no interpolations have been made.

Tineola bisselliella can breed at 33°C. and at 35°C. fertile eggs are able to hatch, but continuous breeding is impossible. Larvae hatched under these conditions never pupate and, although fully grown larvae exposed to this temperature may pupate, the resulting pupae never become adults. Egg-laying is not prevented, but very few viable eggs would be produced as the males are rendered sterile in two days or less.

As far as can be ascertained from the literature, *T. bisselliella* does not occur in tropical climates, though it is otherwise widely distributed throughout the world. This fact may be related to the inability of the moth to withstand temperatures in excess of 33°C. or 34°C. for prolonged periods.

TABLE V.

Lengths of exposures at 70% R.H. required to produce 100% mortality.

		35°C.	36°C.	37°C.	38°C.	39°C.	40°C.	41°C.
Eggs		—	∞	2 days	—	1 day	—	4 hrs.
Larvae	1 day after end of exposure ..	—	—	7 days	18 hrs.	—	3 hrs.	3 hrs.
	10 days after end of exposure ..	—	—	6 days	—	3 hrs.	2 hrs.	—
Pupae		∞	—	—	1 day	—	3 hrs.	—
Adults	1 day after end of exposure ..	6 days	3 days	3 days	1 day	1 day	4 hrs.	—
	5 days after end of exposure ..	5 days	—	—	—	4 hrs.	—	—

∞ = exposure prolonged indefinitely.

The comparatively low temperatures and reasonably short exposure periods necessary to destroy all stages of the insect, point to the possibility of control in the average household by the application of heat to clothing, bedding, furniture, etc. Experiments are in progress to investigate the practicability of raising the temperature of cupboards or small rooms sufficiently to destroy an infestation.

Summary.

All stages of *Tineola bisselliella* were subjected to temperatures between 30°C. and 43°C. for periods varying between a few hours and a number of days.

Although humidity was not an important factor, it was controlled at 70 per cent., and various other humidities were used at some of the lower temperatures.

Complete mortality occurred at relatively low temperatures, 41°C. for four hours being lethal to all stages of the insect.

The eggs appear to be the most resistant stage. Some are able to hatch at 35°C. and some to survive exposure of four hours at 40°C.

Development through the entire life-cycle is possible at 33°C.

Acknowledgements.

This investigation forms part of the research programme of the Pest Infestation Laboratory. The paper is published with the permission of the Department of Scientific and Industrial Research.

The work was suggested by Mr. T. A. Oxley who was then in charge of the Biology Section, and I am indebted to him and to Mr. M. E. Solomon for subsequent discussion and general guidance.

Mr. J. H. Hammond prepared the photographs.

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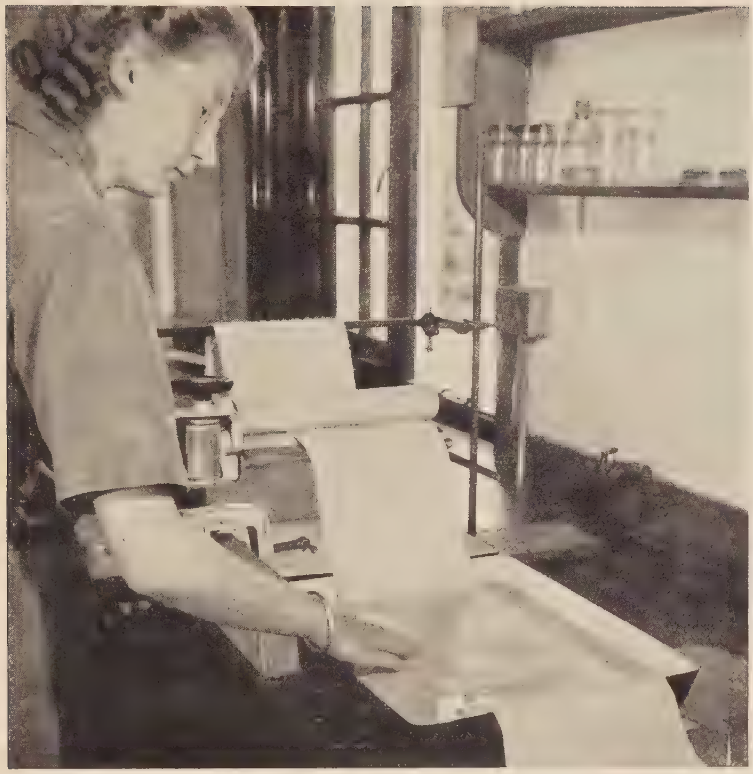


FIG. 1. Preparation of food-impregnated flannel used for rearing stocks of *Tineola bisselliella.*

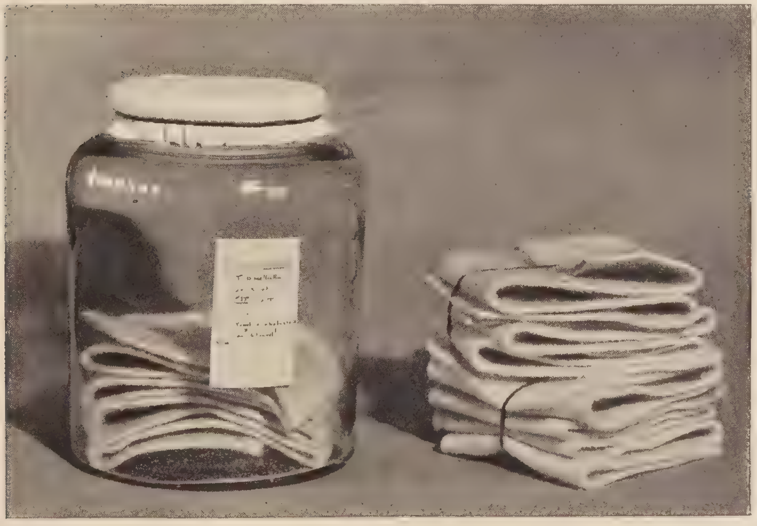


FIG. 2. Impregnated flannel as used in cultures.

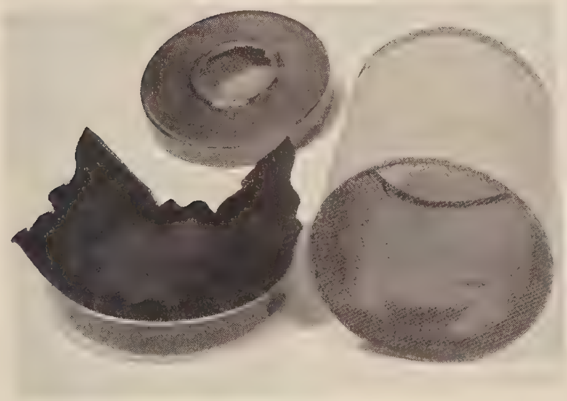


FIG. 1. Parts of oviposition cage.

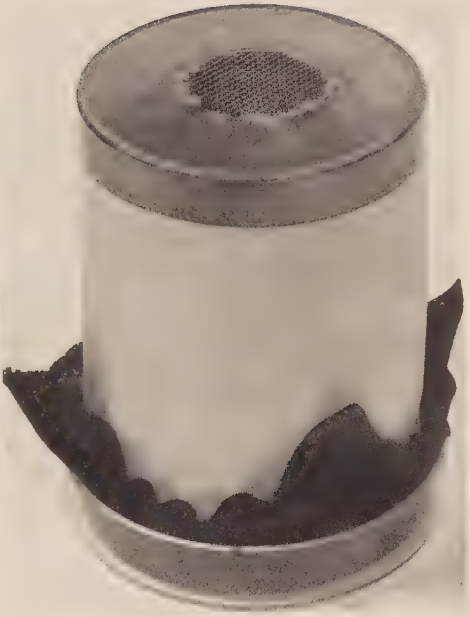


FIG. 2. Oviposition cage assembled.

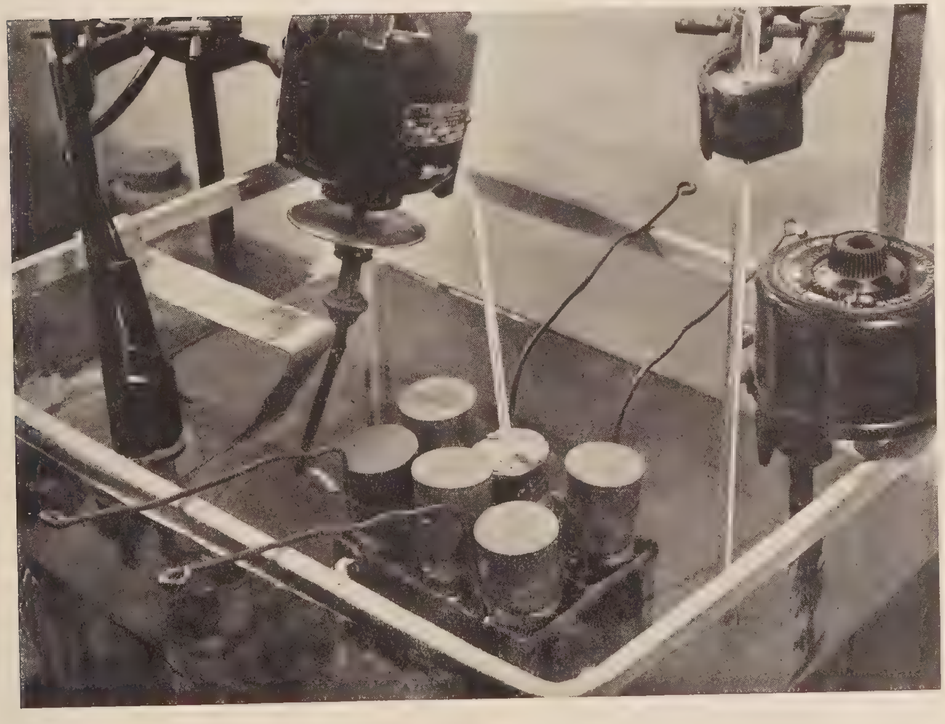


FIG. 3. Controlled temperature water bath.

A NEW GRASSHOPPER (ORTH. ACRIDIDAE) DAMAGING GROUNDNUTS.

By V. M. Dirsh, D.Sc., F.R.E.S.

Anti-Locust Research Centre.

Pyrgomorphella arachidis, **sp. n.**

♂ (*Type*). Of medium size, slender, finely rugulose.

Antenna of 17 segments, shorter than head and pronotum together, thick, slightly flattened and scarcely broadened at the base. Fastigium of vertex long, parabolic, ratio of its length to the width between eyes, 1·8. On the lateral sides of head behind the eyes there is a series of callosities forming a longitudinal stripe, which continues on to the lateral lobe of pronotum.

Pronotum above almost flat, with weakly developed and slightly divergent backward lateral carinae ; its anterior margin truncate, posterior one slightly incurved, two fine transverse sulci ; lower margin of lateral lobe strongly sinuate. Prosternal tubercle broad, low, almost pyramidal, with short acute apex. Mesosternal lobe short, narrow and angulate with incurved hind margin. Mesosternal interspace very large, twice as long as broad, extending well beyond the mesosternal lobes and closely connected with the metasternal interspace, which is twice as broad as long and oval shaped.

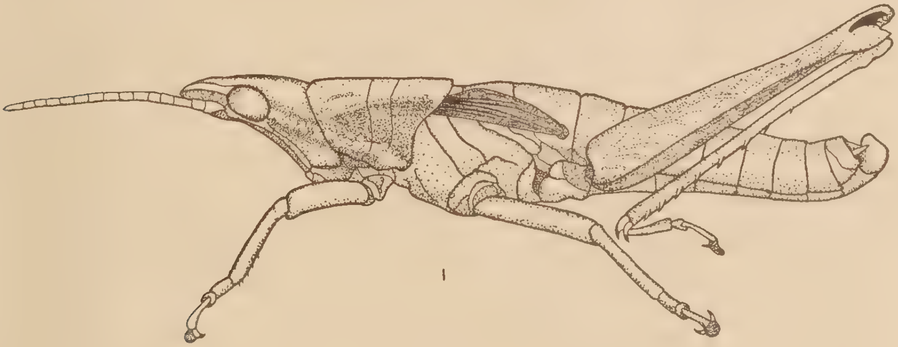


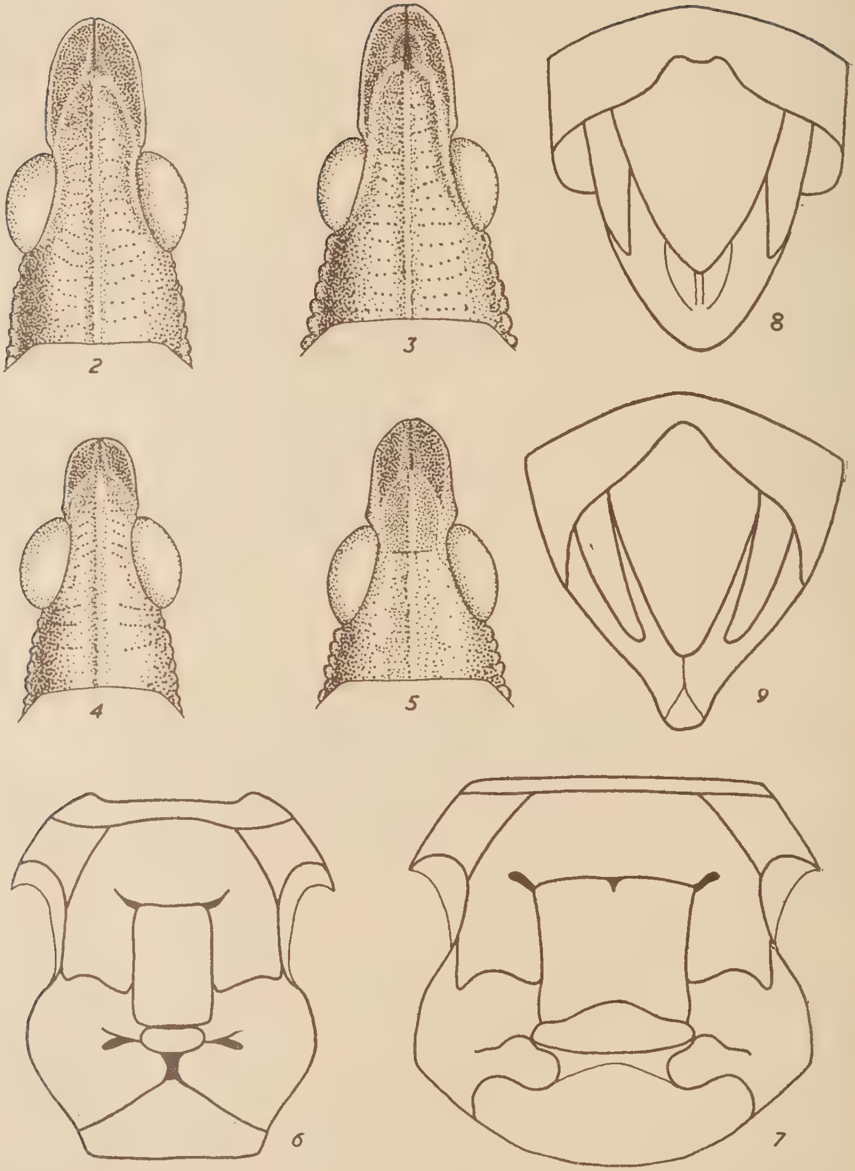
Fig. 1.—*Pyrgomorphella arachidis*, sp.n., male. (× 4.)

Elytron short, lateral, lobiform, reaching and partly covering the tympanum. Wing rudimentary.

Hind femur projects slightly beyond the apex of the abdomen. Hind knee with rounded upper and subacute lower lobe. Tarsal arolium very large, rounded, longer than a claw.

Last abdominal tergite in the middle, with a bi-sinuate excision. Supra-anal plate triangular, with subacute apex ; cercus short, acute. Subgenital plate short subconical.

General coloration green. Antenna grey, with reddish 3rd and 4th basal segments ; lateral stripe along the head and lateral lobe of pronotum yellowish. Hind margin of metanotum red ; base of elytron and the wing red ; along the middle of all abdominal tergites runs a narrow, yellowish stripe. Hind tibia brownish.



Figs. 2–9.—2, *P. arachidis*, head of male; 3, head of female; 6, sternum of male; 7, sternum of female; 8, apex of abdomen; 4, *P. albini*, head of male; 5, head of female; 9, apex of abdomen.

♀ (*Paratype*). Larger and more robust than male. Antenna with 18 segments, the basal segments 3-5th sometimes incompletely divided. Mesosternal interspace large and square. Metasternal interspace four times broader than long.

Supra-anal plate simple triangular. Subgenital plate with a shallow excision at the apex. Ovipositor short, robust, upper valve broad, strongly serrated on the upper margin and with recurved apex; lower valve narrow with obtuse projection on the external margin and decurved apex. In all other characters as the male.

Coloration as in the male.

The species occurs in two colour forms: green or brown. Both forms occur in about equal number in males and females and there are all transitions between them.

Measurements of type in mm.: Length of body 25, pronotum 5·5, elytron 3·5, hind femur 12.

Variations of measurements: length of body ♂ 18–25·5, ♀ 26–31·5; pronotum ♂ 4–5·5, ♀ 5–6; elytron ♂ 2–4, ♀ 4·5–4; hind femur ♂ 8·5–14, ♀ 11·5–13.

Tanganyika Territory: Old Shinyanga, i-ii-iii-iv, 1947, 41 ♂♂ (including type), 28 ♀♀; Mkwemi, 22 miles W. of Kahama, xii, 1946, 5 ♀♀ (*E. Burtt*).

The nearest species to *P. arachidis* is *P. albini* (Chop.) but in *P. arachidis* the fastigium of vertex is much longer and without a projecting lateral margin at the base, and the pronotum is more widened backwards. Elytron in *P. arachidis* is broader and differs in shape from that in *P. albini* in both sexes. In the male, the last abdominal tergite has a bi-sinuate excision, while in that of *P. albini* the excision is simple.

Dr. E. Burtt has supplied the following observations on this new species:

" Serious damage is done only to young plants about two weeks old. Once the plants are established, damage is slight. The pest occurs in great numbers during seasons of heavy rain and may then decimate the entire crop, but when rainfall is low, numbers are insufficient to cause much damage. The damage occurs about December, soon after the groundnuts have been planted. The insect maintains itself on wild herbage, but leaves the latter as soon as the groundnuts are available. At Seke it has been known as a pest for upwards of 30 years. According to African reports, there is only one generation in the year, the new adults appearing during the late rains—about April and May. This seems doubtful, however, and I suspect there are at least two generations in favourable seasons.

" This grasshopper is known to occur over wide areas of the Lake Province and also further West. Seke is about 12 miles north of Old Shinyanga; I know of specimens taken within a few miles of Old Shinyanga in other directions also. I have seen it 35 miles north of Seke, and specimens were caught at Mkwemi, 100 miles to the west of Shinyanga."

I have compared the type of *Parasphena albini* Chop., kindly sent me by Dr. L. Chopard, with *Pyrgomorphella variegata* Key and find them identical. The following is the synonymy:

Pyrgomorphella albini (Chopard)

Parasphena albini Chopard, 1921, Voy. G. Babault Afr. orient., Orth., p. 54, fig. 51.

Pyrgomorphella variegata Key, 1937. Ann. S. Afr. Mus., 32, p. 161, Pl. XVII, A, L. (**SYN. NOV.**)

DISTRIBUTION AND FATE OF *ANOPHELES GAMBIAE* AND *A. FUNESTUS* IN TWO DIFFERENT TYPES OF HUTS TREATED WITH DDT AND BHC IN UGANDA.

By P. R. Wilkinson.

Colonial Insecticides Research Unit, East Africa.

These studies on *Anopheles gambiae* Giles and *A. funestus* Giles, were designed as a repetition in Uganda of the work of Muirhead Thomson (1949). It was found that mud huts of native-type construction and window-traps of cotton mosquito netting were not ideal material to work with, as the thatch and irregularities in the walls made live and possible dead mosquitos difficult to find, and the netting traps were flimsy and difficult to standardise. In addition, ants, which rapidly remove dead mosquitos from the floor, are difficult to exclude from this type of hut. Muirhead Thomson also speaks of entry through minute cracks and crevices and departure, due to a phototaxis, through the window trap, but there is no means mentioned of preventing possible departures through the places of entry and the number escaping in this way can only be estimated by hanging a large muslin net over the hut. For these reasons, wood-and-fibre-board huts were made and used on alternate nights with mud huts of Muirhead Thomson type. The site of the experiment is at the Mpala brickfields, about seven miles from Entebbe on the Kampala road.

A. The Wood-and-Fibre-Board Huts.

Description.

The huts were adapted from the animal baited huts described by Magoon (1935), and like them are of sectional construction. Chief dimensions are: floor 7 ft. by 4 ft. high wall, height 7 ft.; low wall, height 4 ft.; gap in entry and exit traps ¼ in. They were made of local fibre-board on a wooden framework, the chief changes from Magoon's trap being the increase of weatherproofing to make it suitable for human occupation, and the addition of an exit trap. This exit trap was placed to point towards the east and the entry traps were shaded so that a similar effect was obtained to that in Muirhead Thomson's huts, but catching inside the huts was much easier. In order to test whether mosquitos could escape back through the entry trap or cracks, the entry traps of the DDT hut were covered with gauze covers and 44 Anophelines released. The following morning 11 were dead in the exit trap and 33 on the floor; if escape had been possible, a number would either have been missing or would have been found dead in the entry trap.

The huts stood on aluminium-covered legs supported on bricks covered with heavy oil and although there were at times large numbers of ants round the huts, they were only found inside on one occasion, when the oil had been allowed to get dry and become covered with dust.

The walls were of soft fibre-board and were considered to be comparable to wall-plaster of European type or to a less extent African mud wall, when used with dispersible powders.

Spraying.

It was decided to apply the insecticide to a removable lining of fibre-boards, in case the huts should be required for other work at the end of the experiment. The lining boards were cut to shape and then laid on the ground with five sample papers distributed over their surface. These sample papers consist of blotting paper folded twice to a square of four inch side. Guesarol dispersible powder (33 per cent. total DDT, 25 per cent. p, p′) at one pound to the gallon was used for hut No. 2 and I.C.I. DP.530 (50 per cent. crude BHC and 6·5 per cent. gamma) at the same rate was used for hut No. 3. Spraying was carried out personally (African assistants tend to give sample papers an overdose) using a ' Four Oaks ' pressure type sprayer with one of the double nozzles blocked. After spraying, the boards were taken up and fitted into the huts, any small spaces being filled with cotton-wool.

Total DDT and BHC were found by estimating the hydrolysable chlorine in the sample papers, and active isomers calculated from the makers' figures. The average of five papers gave 439 mg./sq. ft. DDT, equivalent to 334 mg./sq. ft. of p, p′, and 273 mg./sq. ft. BHC, equivalent to 35.5 mg./sq. ft. gamma isomer. No. 1 hut was left untreated.

The spraying was carried out on 12th April, 1949.

Catching Routine.

Mosquito searchers slept without mosquito nets, one searcher in each hut. Sheets were spread on the floor about 7 p.m. and the door shut for the night about 10 p.m. At dawn or shortly after, searchers first picked up any dead mosquitos that were readily visible on the sheets, and searched the walls and roof for live mosquitos, using torches and sucking tubes. The floor sheets were then folded up loosely and taken outside for further examination for dead mosquitos. The exit traps were examined for live and dead specimens. Live mosquitos were transferred to small calico cages and dead to specimen tubes, for transport to the laboratory. Each hut had four possible categories—live and dead from inside the hut and live and dead from the exit trap and separate containers were provided for each. Frequent and detailed supervision was essential.

Explanation of table of catches.

Females only are recorded. Male Anophelines were at times numerous in hut No. 1. *Taeniorhynchus* spp. were numerous and troublesome biters but are not recorded. The daily records have been added up into periods of usually six days, as in Muirhead Thomson's Dar-es-Salaam report. As catching took place in these wooden huts, and in the mud huts described later, on alternate nights, six days catches sometimes occupied two or three weeks. In addition to the categories alive ' and ' dead ' represented by A and D, catches were further sub-divided, classified as ' fed ' or ' unfed ' : fed mosquitos were those which had fed the night before, as shown by the presence of red undigested blood in the abdomen. When a dash is shown in the table, it indicates that no sheets were on the floor, so that dead mosquitos in the huts were not recorded. The single vertical line marks the time of spraying.

TABLE I.

Catches in treated and untreated wood- and fibre-board huts.

Hut No. 1. Untreated control.

A. gambiae.

No. of Collections			5	6	6	4	6	7	6	6	6	6	6	6	2
No. of weeks before or after spraying			13 to 12	12 to 11	10 to 9	9	5 to 4	4 to 1	1 to 3	3 to 5	6 to 8	8 to 10	11 to 13	13 to 15	16
Inside hut	Fed	A	13	29	16	15	10	13	14	29	21	44	36	32	6
		D	—	—	0	0	0	0	0	0	0	0	0	0	0
	Unfed	A	4	7	12	6	18	14	3	4	17	6	2	2	1
		D	—	—	0	0	0	0	0	0	0	0	0	0	0
In exit trap	Fed	A	32	56	43	26	10	15	7	1	3	5	6	0	0
		D	0	0	0	0	0	0	0	0	0	0	0	0	0
	Unfed	A	2	13	17	10	16	10	1	3	2	7	4	1	1
		D	0	0	0	0	0	0	0	0	0	0	0	0	0
		A. funestus.													
Inside hut	Fed	A	14	13	14	15	16	7	9	26	25	52	39	81	18
		D	—	—	0	0	0	0	0	0	0	0	0	0	0
	Unfed	A	2	17	21	16	21	14	7	10	11	10	9	13	2
		D	—	—	0	0	0	0	0	1	0	0	0	0	0
In exit trap	Fed	A	19	34	37	28	2	9	8	3	12	4	8	2	0
		D	0	0	0	0	0	0	0	0	0	0	0	0	0
	Unfed	A	7	15	46	23	5	7	5	4	10	11	8	4	3
		D	0	0	0	0	0	0	0	0	0	0	0	0	0

Hut No. 2. 332 mg./sq. ft. p, p′ DDT.

A. gambiae.

No. of Collections			2	6	7	6	6	6	6	6	6	2
No. of weeks before or after spraying			9	5 to 4	4 to 1	1 to 3	3 to 5	6 to 8	8 to 10	11 to 13	13 to 15	16
Inside hut	Fed	A ..	9	4	15	0	0	0	0	0	0	0
		D ..	0	0	0	7	7	7	13	9	7	1
	Unfed	A ..	1	9	9	0	0	0	0	0	0	0
		D ..	0	0	0	1	14	8	8	3	3	0
Exit trap	Fed	A ..	3	6	20	0	0	0	0	0	0	0
		D ..	0	0	0	1	0	2	4	3	1	0
	Unfed	A ..	0	4	9	0	2	1	0	0	0	0
		D ..	0	0	0	1	1	0	2	2	0	0
		A. funestus.										
Inside hut	Fed	A ..	2	10	25	0	0	0	0	0	0	0
		D ..	0	0	0	7	11	6	17	20	25	8
	Unfed	A ..	5	8	12	0	0	0	0	0	0	0
		D ..	0	0	0	2	12	11	13	12	20	15
Exit trap	Fed	A ..	1	7	6	0	0	0	0	0	0	0
		D ..	0	0	0	2	0	2	1	1	2	0
	Unfed	A ..	1	4	6	0	0	0	0	0	0	0
		D ..	0	0	0	0	4	1	2	3	2	4

Hut No. 3. 35·5 mg./sq. ft. gamma BHC.

A. gambiae.

No. of Collections			6	7	6	6	6	6	6	6	2
No. of weeks before or after spraying			5 to 4	4 to 1	1 to 3	3 to 5	6 to 8	8 to 10	11 to 13	13 to 15	16
Inside hut	Fed	A	11	11	0	0	0	0	0	0	0
		D	0	0	4	1	6	8	7	1	0
	Unfed	A	6	7	0	0	0	0	2	0	0
		D	0	0	2	8	6	12	4	1	0
Exit trap	Fed	A	9	9	0	0	0	0	0	0	0
		D	0	0	0	0	1	1	0	0	0
	Unfed	A	7	8	0	0	0	0	0	0	0
		D	0	0	0	0	1	0	0	0	0
A. funestus.											
Inside hut	Fed	A	10	23	0	0	0	0	0	0	0
		D	0	0	3	6	5	5	4	5	0
	Unfed	A	6	10	0	0	0	0	3	0	0
		D	0	0	1	10	11	7	8	21	15
Exit trap	Fed	A	11	15	0	0	0	0	0	0	0
		D	0	0	0	0	1	0	0	0	0
	Unfed	A	7	10	0	0	0	0	0	0	0
		D	0	0	0	0	3	2	0	0	1

Meteorological Data.

These are taken from Entebbe airfield, about six miles away.

	Jan.	Feb.	Mar.	Apr.	May	June	July
Mean maximum temperature °F.	81·0	79·2	82·1	78·8	77·5	77·2	76·1
,, minimum temperature °F...	62·4	63·0	64·7	64·7	63·4	61·7	60·9
Rainfall in ins.	3·42	3·95	2·45	10·0	3·89	3·56	2·15
,, No. of days	5	10	9	20	10	6	11

Discussion.

The catches may be summarised to give information on three main points (a) the proportion of mosquitos killed by the insecticide, (b) the proportion feeding, (c) the proportion in the exit trap. There are other categories formed by combination of these factors, for instance the number of fed mosquitos alive in the exit cage is of interest, since it represents possible survivors capable of transmitting a malaria infection from an infected hut occupant. However, on examining the figures from the untreated hut we find that the proportions feeding and remaining inside vary even in the absence of insecticide, so that it is difficult to relate these states and actions to the application of the insecticide. In the untreated hut, there was some evidence that the total number entering the hut and the proportion entering the exit trap, were dependent on weather conditions, but further work would be required to decide this.

A simple form of summary is provided by the percentage of all mosquitos collected which were dead in huts nos. 2 and 3 over the treated period of 16 weeks (Table II). Apart from the one dead *A. funestus* mentioned on p. 50, no dead mosquito was found in the control hut (no. 1).

TABLE II.

Totals of mosquitos collected in treated wooden huts, and percentage kill.

Species	Hut No.	Treatment	No. alive	No. dead	% kill	Situation of live mosquitos
A. gambiae ..	2	DDT	3	103	97	In exit trap
A. funestus ..	2	DDT	0	203	100	—
A. gambiae ..	3	BHC	2	66	97	Inside hut
A. funestus ..	3	BHC	3	108	97	„ „

The survival of live Anophelines in the BHC treated hut was unexpected, from the literature. Statements by the African occupants indicated that they may have entered at dawn or shortly before, and in that case would have had a relatively short contact time with the insecticide. A possible contributory factor was that the wooden huts became perceptibly warm during hours of sunshine and it is known that the decomposition of BHC is rapid at high temperatures (Progr. Rep. Colon. Insect. Res. Comm. no. 3, 1947, p. 10).

It is of interest to compare similar figures extracted from Muirhead Thomson (4th Quart. Progr. Rep. Malar. Invest. Med. Lab., Dar-es-Salaam, 1948) based on observations in mud huts over a period of five weeks for the DDT hut and ten weeks for the BHC hut.

TABLE III.

Totals of mosquitos collected in treated mud huts, and percentage kill. (Muirhead Thomson, 1948.)

Species	Hut treatment	No. alive	No. dead	% kill	Situation of live mosquitos
A. gambiae ..	DDT	553	11	2	In exit trap
A. gambiae ..	BHC	0	740	100	—

The original figures also show 98 per cent. of the *gambiae* entered the exit trap in the DDT hut, whereas in the BHC hut 9·4 per cent. entered the exit trap and all of these died.

The DDT dosage used by Muirhead Thomson was 400 mg./sq. ft. of the powder (method of assessment not stated). This is believed to correspond to 100 mg./sq. ft. of p, p′ DDT, which is about one-third of the dosage used in hut No. 2. While the dosage used by Muirhead Thomson is probably nearer that which would be used in practice, the figures obtained in hut 2 show that DDT at the heavier dosage used in the present experiment gives a high kill of *gambiae* and a higher kill of *funestus*. The BHC dosage used by Muirhead Thomson was approximately 24 mg./sq. ft. of gamma isomer, which is 0.69 of the dosage in hut 3.

If the total catches for the huts (Table IV) are examined it appears that a drop occurs in the catches after treatment, particularly in the BHC treated hut :—

TABLE IV.

Totals of mosquitos collected in control and treated wooden huts, before and after treatment.

Period before or after spraying		—2	—1	+1	+2	+3	+4	+5	+6	+7
Species	Hut No.									
A. gambiae ..	1	54	52	25	37	43	62	46	35	8
,, ..	2	23	53	10	24	18	27	17	11	1
,, ..	3	35	35	6	9	14	21	13	2	0
A. funestus ..	1	44	37	29	44	58	77	64	99	23
,, ..	2	29	49	11	27	20	33	36	49	27
,, ..	3	34	58	4	16	20	14	15	26	16

This drop in catch would seem to be due to reduced entry, since unrecorded escapes seem unlikely from the construction of the huts and the experiment described earlier. A reduced entry might be due either to a distant repellent effect of the insecticide or the masking of the attractive human odour by the insecticide odour.

The presence of one dead *funestus* in the untreated hut 3 to 5 weeks after spraying is presumed to be adventitious.

B. The Mud Huts.

Description.

These were made to follow the description in Muirhead Thomson (1948), the window trap being copied as far as possible from the photograph. The huts were made circular instead of square in plan, and about 7 ft. in diameter. The only additions were a ditch about 1 ft. deep surrounding each hut, with an inner concrete channel of semi-circular cross section and diameter 3 ins. The inner channel was about 1 ft. from the hut wall and the ditch about 2 ft. from the channel. The concrete channel was kept filled with water and earlier in the experiment the water was covered with malariol or other spreading oil, but the use of oil was later abandoned in case of any possible repellent effect on mosquitos. The water-filled channel was to stop ants wandering into the huts from outside. The 1 ft. ditch was intended to produce a drying out of the hut floor and so discourage burrowing ants and termites. It may be noted that termites were troublesome in the untreated hut but not in the treated huts.

Ants.

Immediately after building there was a large ant population in the huts, particularly huts 4 and 5. Pyrethrum spraying, treatment of nest-holes with oil and hand catching failing to effect a sufficient reduction, it was decided to use a residual insectitide on these two huts as it was of the first importance to eliminate the ants and frequent use of pyrethrum/kerosene sprays might have reduced mosquito entry. The residual spray could be limited to the floor which would be covered with sheets, and any chance contacts of mosquitos with the floor would be improbable and unimportant compared with the wall and roof treatment. In addition, it is common experience that a considerable quantity of insecticide falls to the floor when spraying hut roofs and walls. Accordingly, a light spraying with Guesarol 33 at 1 lb. to the gallon was given to the floors of huts 4 and 5 in December, 1948, approximately 17 weeks before the main spraying. This reduced the ants, which were again reduced by the main roof and wall spraying. The effect on the Anopheline population of the floor spraying appeared to be nil, as there were no deaths before the main spraying, except a solitary *funestus* in the exit trap of No. 5 hut, and a similar isolated death has been noted in the untreated wooden hut. Ants in the untreated mud hut No. 6 were kept down by hand catching and occasional spraying with 0·1 per cent. pyrethrins in kerosene.

Spraying.

The formulations and apparatus were the same as for the wooden huts, and spraying was carried out on the same day. Two sample papers were placed on the roof and three on the walls. The averages of five papers gave 193 mg./sq. ft. DDT (145 mg./sq. ft. p, p′) for hut 4 and 212 mg./sq. ft. BHC (27·5 mg./sq. ft. gamma) for hut 5. These are equivalent to 1·4 and 1·1 times the dosage used by Muirhead Thomson.

Catching Routine.

This was essentially the same as in the wooden huts. In addition to the searcher in each hut, another man slept in the hut, his function being to increase the attractiveness of the hut to mosquitos ; it is known that, for a given hut, mosquito entry increases, within limits, with the number of occupants.

Before treatment most of the mosquitos in the huts were found at the junction of wall and thatch. A laboratory stool was provided, so that the thatch could be inspected, and grass brushes for use as described by Muirhead Thomson, but few mosquitos were seen on the higher parts of the thatch.

Catches are recorded in the same manner as for the wooden huts.

TABLE V.

Catches in treated and untreated mud-and-thatch huts.

Hut No. 4. 145 mg./sq. ft. p, p′ DDT.

A. gambiae.

No. of Collections			6	6	6	6	6	6	6	6	6	6	2
No. of weeks before or after spraying			12 to 11	10 to 9	6 to 5	3 to 2	1 to 3	3 to 5	6 to 8	9 to 11	11 to 13	14 to 16	16
Inside hut	Fed	A	25	17	20	12	0	0	0	0	0	0	0
		D	—	0	0	0	1	1	1	2	0	0	0
	Unfed	A	13	21	18	9	0	0	0	0	0	0	0
		D	—	0	0	0	2	3	6	3	1	1	0
Exit trap	Fed	A	10	11	13	26	1	0	2	0	1	0	0
		D	0	0	0	0	0	0	0	0	0	0	0
	Unfed	A	7	14	12	17	0	3	0	6	2	0	0
		D	0	0	0	0	2	1	0	0	0	0	0

A. funestus.

Inside hut	Fed	A	13	15	10	6	0	0	0	0	0	0	0
		D	—	0	0	0	0	2	2	2	0	1	0
	Unfed	A	11	31	17	15	0	0	0	0	0	0	0
		D	—	0	0	0	0	3	8	6	2	7	1
Exit trap	Fed	A	5	6	8	11	0	0	0	0	0	0	0
		D	0	0	0	0	0	0	0	0	0	0	0
	Unfed	A	9	16	5	19	0	6	2	3	1	0	0
		D	0	0	0	0	4	1	2	0	1	0	0

Hut No. 5. 27·5 mg./sq. ft. gamma BHC.

A. gambiae.

No. of Collections			6	6	6	6	6	6	6	6	6	6	2
No. of weeks before or after spraying			12 to 11	10 to 9	6 to 5	3 to 2	1 to 3	3 to 5	6 to 8	9 to 11	11 to 13	14 to 16	16
Inside hut	Fed	A	31	20	8	20	0	0	0	0	0	0	0
		D	—	0	0	0	5	2	2	1	0	0	0
	Unfed	A	0	14	6	3	0	0	0	0	0	0	0
		D	—	0	0	0	1	4	13	4	3	2	0
Exit trap	Fed	A	11	1	8	30	0	0	1	0	0	0	0
		D	0	0	0	0	2	0	2	0	0	1	0
	Unfed	A	2	10	5	9	0	0	0	0	0	0	0
		D	0	0	0	0	1	3	3	1	1	0	0

A. funestus.

Inside hut	Fed	A	29	29	8	11	0	0	0	0	0	0	0
		D	—	0	0	0	8	5	4	9	2	4	1
	Unfed	A	23	27	12	8	0	0	0	0	0	0	0
		D	—	0	0	0	4	6	12	5	4	8	5
Exit trap	Fed	A	2	5	5	26	0	0	0	0	0	0	0
		D	0	0	0	0	0	0	0	0	0	0	0
	Unfed	A	3	11	17	11	0	0	1	1	0	4	0
		D	0	0	1	0	0	5	3	2	0	0	1

Hut No. 6. Untreated control.

A. gambiae.

No. of Collections			6	6	6	6	6	6	6	6	2
No. of weeks before or after spraying			6 to 5	3 to 2	1 to 3	3 to 5	6 to 8	9 to 11	11 to 13	14 to 16	16
Inside hut	Fed	A	9	47	14	9	4	1	2	0	1
		D	0	0	0	0	0	0	0	0	0
	Unfed	A	10	7	2	2	1	3	1	0	0
		D	0	0	0	0	0	0	0	0	0
Exit trap	Fed	A	1	22	8	12	9	2	1	0	0
		D	0	0	0	0	0	0	0	0	0
	Unfed	A	13	8	3	7	14	14	1	1	0
		D	0	0	0	0	0	0	0	0	0

A. funestus.

Inside hut	Fed	A	8	15	24	8	0	9	5	4	3
		D	0	0	0	0	0	0	0	0	0
	Unfed	A	3	12	7	1	4	15	0	6	5
		D	0	0	0	0	0	0	0	0	0
Exit trap	Fed	A	4	14	20	15	9	1	3	2	1
		D	0	0	0	0	0	0	0	0	0
	Unfed	A	7	9	4	17	38	29	12	8	9
		D	0	0	0	0	0	0	0	0	0

Discussion.

The impression gained from Muirhead Thomson's paper was that almost all the *gambiae* in a DDT hut were found alive, fed, and mostly in the exit trap, while in the BHC hut they were almost all dead inside the hut. This implies that for practical purposes DDT would be ineffective while BHC would be very effective.

The figures from our mud huts (Table VI), with dosages closely similar to those used in Tanganyika, do not give such a wide difference between the performance of the two insecticides, though over the period BHC appeared to be the more effective. It is hoped that observations will continue to study the durations of the residual effects.

TABLE VI.

Totals of mosquitos collected in treated mud huts, and percentage kill.

Species	Hut No.	Treatment	No. alive	No. dead	% kill	Situation of live mosquitos
A. gambiae ..	4	DDT	15	24	62	In exit trap
A. funestus ..	4	,,	12	42	78	,, ,,
A. gambiae ..	5	BHC	1	51	98	,, ,,
A. funestus ..	5	,,	6	86	93	,, ,,

As in the treated wooden huts, the total catches in the treated mud huts (Table VII) were lower after spraying.

TABLE VII.

Totals of mosquitos collected in control and treated mud huts, before and after treatment.

Period before or after spraying		—2	—1	+1	+2	+3	+4	+5	+6	+7
Species	Hut No.									
A. gambiae ..	6	33	84	27	40	28	20	5	1	1
,, ..	5	27	61	9	9	21	6	4	3	0
,, ..	4	63	64	6	8	9	11	4	1	0
A. funestus ..	6	22	50	55	41	51	54	20	20	18
,, ..	5	43	56	12	16	20	17	6	16	7
,, ..	4	40	51	4	12	14	11	4	8	1

The reduction may be due to reduced entry, as in the wooden huts, but escapes through eave cracks are not impossible; as it has been pointed out, this is a weakness inherent in the primitive construction of the mud huts.

Summary.

Wood-and-fibre-board huts fitted with one-way entry and exit traps were sprayed with DDT and BHC wettable powders at dosages 3·3 and 1·5 times the dosages reported by Muirhead Thomson in Tanganyika. Mud huts similar to his design were sprayed at 1·4 times his dosage of DDT and 1·1 times his dosage of BHC. Catches of Anopheline mosquitos continued for 16 weeks.

In the first-named huts kills of *A. gambiae* were 97 per cent. for both treatments. Kills of *A. funestus* were 100 per cent. and 97 per cent. for DDT and BHC respectively.

In the mud huts kills of *A. gambiae* were 62 per cent. and 98 per cent. for DDT and BHC respectively. For *A. funestus* kills were 78 per cent. and 93 per cent. in the same order.

The advantages claimed for the wood-and-fibre-board huts include :—

(a) freedom from ants, which may remove dead mosquitos.
(b) ease of catching live adult mosquitos.
(c) rigid construction of exit trap.
(d) lack of adventitious exits other than exit trap.

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Note by K. S. Hocking.

Since this paper was written, serious differences between the DDT wettable powder used by Muirhead Thomson (Shell Ditrene) and that used by Wilkinson (Guesarol 33) have come to light.

The Ditrene contains a much larger proportion of large particles of DDT which would (a) separate out in the drums of powder so that a small sample might not be representative and (b) settle rapidly after mixing with water so that the dosage applied to the walls (which was not estimated chemically) may have been much less than Muirhead Thomson supposed.

Further, recent experiments of Hadaway and Barlow (in press) show that the large particles of DDT in Ditrene are much less toxic to mosquitos than the smaller ones in Guesarol.

Most of the failure to kill found by Muirhead Thomson was therefore probably due to a low dosage of a poor formulation.

Further experiments are in progress to elucidate this point.

FURTHER OBSERVATIONS ON THE EXTERMINATION OF *GLOSSINA PALPALIS FUSCIPES* NEWSTEAD BY HAND-CATCHING.

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(Plate IV.)

The successful extermination of *Glossina palpalis fuscipes* Newst. (referred to hereafter as *G. palpalis*) in Kenya on a small river with a narrow strip of riverine bush and a low density of *G. palpalis* has already been reported (Glasgow & Duffy, 1947). The areas previously treated are still free of *G. palpalis*, but an attempt to extend the method to a large river with a wider strip of forest and a medium density of *G. palpalis* has been unsuccessful.

As recorded in our previous paper, *G. palpalis* was "caught out" in the Experiment Block of the Sari River for seven months ending December, 1945, and in the Control Block of the Sari River for six months ending May, 1947. The blocks were examined again at the end of April, 1949, and for eight working days, 30 fly-boys patrolled the Experiment Block and 27 fly-boys patrolled the Control Block without catching or seeing any tsetse.

Experiment on the Kuja River.

Location of the experiment.

The Sari runs into the Kuja River, which is a permanent stream 20 to 30 yards wide in dry weather. Its banks carry forest, more luxuriant than the Sari bush. The width of the forest depends upon the width of flat land bordering the river and it is about 150 yards wide some six miles below the confluence with the Sari. The Kuja meanders from side to side of this forest, some of its bends actually emerging into the surrounding grassland. A block of a mile and a half of this forest, known as Block VII, was the scene of the second hand-catching experiment (see fig. 1, and Plate IV, fig. 1).

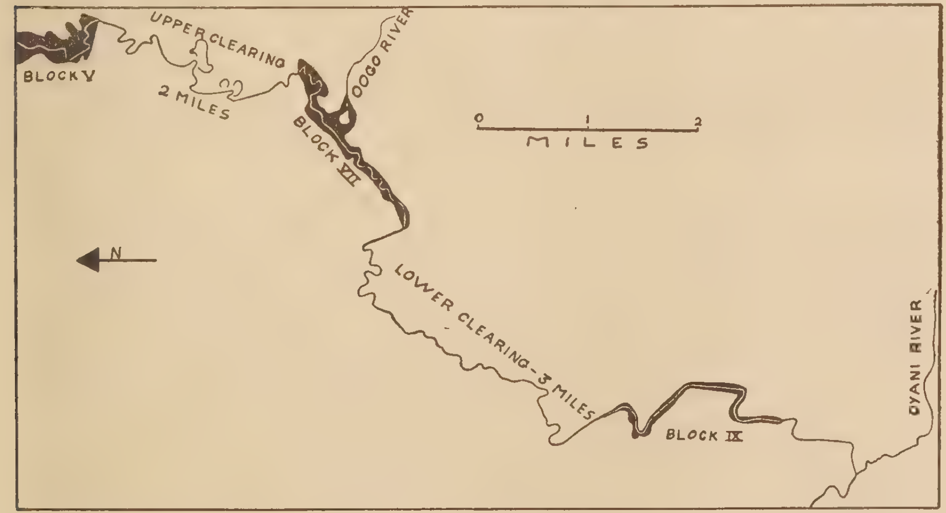

Fig. 1.—Kuja River, showing Block V, Block VII (experiment) and Block IX (control).

A small seasonal tributary, the Oogo, joins the Kuja on its left bank near the top end of Block VII. Block VII is 400 yards wide at its top end because of the fusion of the Oogo bush and the Kuja bush, although in general the width is about 150 yards. The latter figure is the width of forest carried by the unaided Kuja in this locality. There are a number of seasonal ponds in the forest. These lie in old beds of the Kuja for, at various times in the past, the Kuja appears to have occupied alternative channels to those in which it now flows. When the Kuja alters its course, roughly the following sequence of events takes place ; this sequence has been deduced from an examination of numerous ponds but such an alteration has not actually been observed. First the new pond is invaded by *Ficus capreifolia* Del., a moisture-loving shrub that forms dense thickets on the inner bank of every bend of the river (see fig. 2, and Plate IV, fig. 2). The deepest part of the pond holds water for most of the year, and here open water continues to exist for a long time, as *F. capreifolia* cannot endure completely aquatic conditions (fig. 2, top left). Gradually, however, the pond silts up, and *F. capreifolia* occupies the whole of it. Further silting leads to the replacement of the *F. capreifolia* thicket by a thicket of *Rhus inamoena* Standly and *Allophylus africanus* P. Beauv. (fig. 2, top right). A continuance of the silting leads to the replacement of the *Rhus-Allophylus* thicket by a fine even stand of *Pachystela brevipes* (Bak.) Engl. (fig. 2, middle bottom). These trees are 50 to 60 feet high, and there is scarcely any undergrowth beneath them (Pl. IV, fig. 5). The pond is by now a barely discernible depression, but it fills when the Kuja is in high flood and holds water for a few weeks. The slightly higher ground that occurs further in at the centre of the forest is scarcely ever flooded. This supports an apparently permanent forest dominated by *Albizzia zygia* (DC) Macbride (Pl. IV, fig. 4). *A. coriaria* Welw. is also found, although this is a savannah tree perhaps more at home outside the riverine forest, in the grassland. The relationships of these various

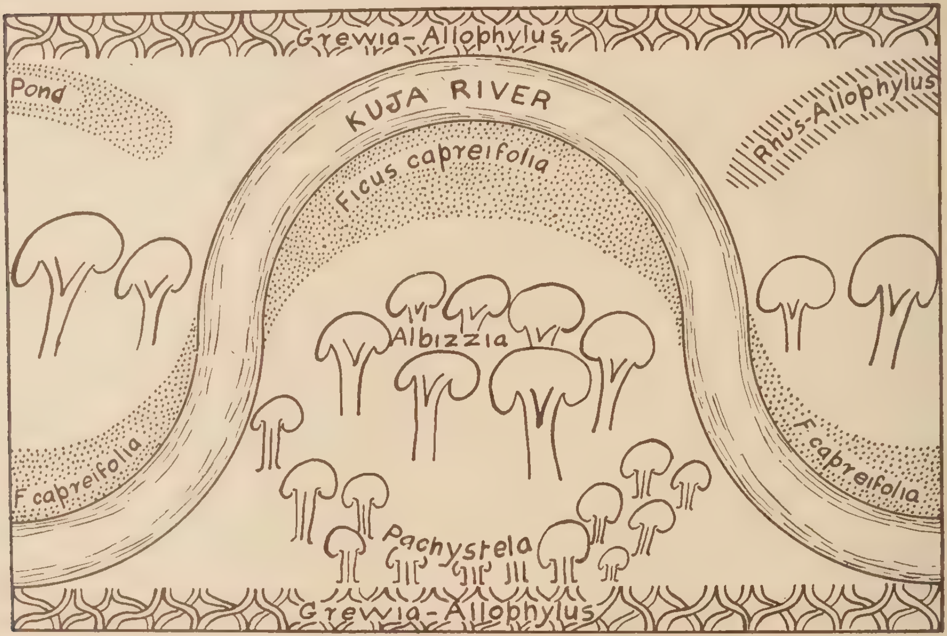

Fig. 2.—Diagram of various vegetation communities comprised in Block VII.

vegetation associations are shown in fig. 2, which is a diagrammatic map representing part of the lower end of Block VII. In the upper, wide part of Block VII the same communities are recognisable, although a more complicated topography produces a more complicated appearance. The transition from grassland to forest is exceedingly abrupt, the edge of the forest being sealed by an impenetrable thicket of which the most conspicuous components are *Grewia* sp. and *Allophylus africanus*, although almost any species found in the forest is sometimes seen in the fringing thicket. It must not be supposed that the seven mentioned above are by any means the only species found in the block—they are those characteristic of the various associations.

Block VII was separated from other Kuja riverine forest by large clearings in which no woody growth and no long grass was tolerated. The upstream clearing was two miles long and the downstream clearing three miles (fig. 1), later extended to three and a half miles.

TABLE I.

Cost of clearing in men-days.

	Matchets	Axes	Firebreaks	Burning	Planting Grass	Total
Upper Clearing ..	6,953	6,332	514	1,058	1,268	16,125
Lower Clearing ..	3,183	6,785	433	865	1,106	12,372

Approximately 100 acres of riverine forest was removed to make each of these clearings. Table I gives the cost in men-days of the various operations involved. Such overheads as the construction of motor tracks and labour camps are omitted from the table. African supervision, at the rate of about 5 per cent. or one headman to every 20 labourers, is also omitted, but the cost of the fire-breaks is included. These were cut in the grassland surrounding the cleared areas, and they serve a double purpose. They prevented the felled bush from being ignited too early by casual grass fires and, when burning was in progress, they prevented the fires from spreading and causing damage to the neighbourhood. The clearings were begun in 1945 and completed in 1946. An average of 217 men-days per month were spent on maintenance of the upper clearing in 1947, and of 165 men-days on that of the lower clearing. It is to be remembered that these were isolation clearings and as such were maintained meticulously.

The operation of planting grass, the cost of which (Table I) was 8 per cent. of the total cost of the clearings, is regarded as very important. The grass planted was *Cynodon* sp. a low (knee-high) creeping grass which prevents soil erosion. *Cynodon* can only be established by planting pieces of it which include some root. These were normally planted immediately after the first rain which fell after burning. The bits of grass are planted one pace apart in rows one pace apart. If *Cynodon* is not established the clearings are colonized by free-seeding grasses, some of which, such as *Panicum maximum* and *Sorghum verticilliflorum*, are undesirable because they grow ten feet tall, and do not bind the soil as effectively as *Cynodon*.

At the bottom of the downstream clearing there was a stretch of riverine forest, Block IX, of about the same length as Block VII. This was demarcated by another large clearing below it, and was used as a control for the Block VII experiment. Table II gives the results of fly rounds carried out in Block IX during the period of the experiment.

TABLE II.

Block IX Fly-round. Control for Block VII experiment.

(The figures in brackets after the names of the months indicate the number of rounds done in each month.)

Date	Teneral %	Non-Teneral Female %	Non-Teneral Males per 10,000 yards
1946			
July (4)	39	28	137
Aug. (5)	29	23	122
Sept. (4)	28	25	116
Oct. (4)	25	16	107
Nov. (5)	37	17	115
Dec. (4)	47	16	80
1947			
Jan. (5)	46	20	90
Feb. (4)	39	17	80
Mar. (4)	51	28	82
Apr. (4)	47	30	110
May (5)	47	41	57
June (4)	48	31	62
July (4)	41	11	85
Aug. (5)	36	11	95
Sept. (4)	35	24	130
Oct. (3)	30	30	142
Nov. (4)	27	26	182
Dec. (3)	28	29	168
1948			
Jan. (5)	36	32	130
Feb. (4)	26	26	115
Mar. (4)	20	29	165
Apr. (5)	28	38	230
May (4)	26	39	170
June (4)	28	37	167
July (5)	27	25	200
Aug. (4)	28	27	392
Sept. (4)	26	23	270
Oct. (5)	33	36	217
Nov. (4)	39	29	270
Dec. (5)	38	25	200
1949			
Jan. (4)	33	40	122
Feb. (4)	24	39	152
Mar. (4)	45	47	30
Apr. (3)	31	61	43

It is sometimes suggested that the removal of riverine forest promotes soil erosion. The rate at which the Kuja eroded its banks was measured at a number of points in the clearings and in Block VII over a period of two years, and no differences were detected. These observations have been fully reported elsewhere (Glasgow & Duffy, 1949).

The hand-catching procedure.

Preliminary observations, which were confirmed during the course of experiment, showed that it was only worth while looking for *G. palpalis* alongside water. Paths were therefore made along the banks of the Kuja and Oogo, and beside all the ponds mentioned above. As soon as the ponds dried up, *G. palpalis* ceased to be caught on the pond paths.

The catchers were organised in teams of three in the Sari experiment, each team carrying a hessian screen and a small cage in which the flies were placed alive as they were caught. Observations carried out since then have, however, failed to show that these practices increased the catch. In the present experiment, therefore, the catchers were organized in teams of two, of which about 26 teams worked each day. These were divided as before into the morning shift, working from 7 till 3, and the afternoon shift working from 11 o'clock till dusk. Various devices were used to ensure that the catchers were at all times spread out as evenly as possible over the block. Table III gives the catches made during the first phase of the experiment, which lasted 48 weeks. By the end of this time it was obvious that no further reduction in numbers was being obtained.

TABLE III.

First phase of Block VII hand-catching experiment.

4 weeks ending	Fly-boy-days	Teneral		Non-Teneral		Grand Total
		Males	Females	Males	Females	
10. 8.46	1,036	672	715	1,521	1,770	4,678
7. 9.46	1,024	935	805	237	368	2,345
5.10.46	1,123	679	524	128	265	1,596
2.11.46	1,448	268	162	90	111	631
30.11.46	1,523	163	93	51	92	399
28.12.46	1,113	86	81	37	46	250
25. 1.47	1,335	128	94	62	49	333
22. 2.47	1,272	59	43	55	35	192
22. 3.47	1,590	114	44	47	31	236
19. 4.47	1,371	78	46	36	35	195
17. 5.47	1,239	30	27	24	51	132
14. 6.47	1,316	9	12	33	57	111

In July and August, 1946, a rowing boat was available, that held two catchers, including the oarsman. The river was divided into four sections each 1,100 yards long and the boat was operated in a different section of the river every day. The boat catch is compared in Table IV with the total catch made by four land catchers (two on each bank) who were working in the same section as the boat. Although the boat held only half as many catchers as were working on land, it caught twice as many flies. This relation was true of all components of the catch ; for example the boat took 68 per cent. of the non-teneral females and 71 per cent. of the teneral females. In September, two local canoes were acquired and in succeeding months these two boats continued to take more than their fair share of the total catch.

TABLE IV.

Comparison of land and water catches.

Section	No. days	Land (4 catchers)	Water (2 catchers)
A	6	86	174
B	11	84	217
C	12	59	132
D	10	117	172
	39	346 33%	695 67%

On the strength of this observation it was decided, when it became obvious that the first phase (Table III) was not leading to success, to put all the riverside catchers into boats. Sixteen small punts, each to hold two catchers, were built, as it was impossible to get so many local canoes. Four punts were assigned to each section and the land catchers now worked only the ponds and tributaries. The second phase began (Table V) on 16–vi–47. A pair of punt-borne catchers is shown in Pl. IV, fig. 2.

TABLE V.

Second phase of Block VII hand-catching experiment.

4 weeks ending	Fly-boy-days	Teneral		Non-Teneral		Grand Total
		Males	Females	Males	Females	
12. 7.47	1,523	43	41	42	37	163
9. 8.47	1,531	54	27	33	17	131
6. 9.47	1,556	57	29	25	27	138
4.10.47	1,691	35	33	20	10	98
1.11.47	1,716	52	25	20	12	109
29.11.47	1,674	50	35	49	23	157
27.12.47	1,519	38	27	39	30	134
24. 1.48	1,594	78	50	40	31	199
21. 2.48	1,530	62	34	44	65	205
20. 3.48	1,256	64	38	36	30	168
17. 4.48	1,258	60	71	51	56	238

Catching continued for a week after 17.4.48 with the following results :—

	244	13	14	20	9	56

Note.—This table refers to catches on land as well as those on the river. Actually in each period of 4 weeks, 768 fly-boy-days were used in the 16 punts, and the variable remainder on land, but the majority of the flies were taken by the punt-borne catchers. Thus 1,408 of the grand total of 1,796 were taken by the punts.

It became clear towards the end of 1947, as will be explained later, that flies were crossing the clearings into Block VII in sufficient numbers to account for the failure to achieve eradication. Attempts (described below) were made to prevent this, but they were unsuccessful and the catching was stopped on 24–iv–48.

After a period devoted to the preparation of further devices for stopping up the clearings, catching out was resumed on 13–xii–48 (Table VI) but it soon became clear that no progress was being made and the experiment was finally closed down on 16–iv–49.

Passage of G. palpalis *across the clearings.*

Flies were marked in Block VII, as a preliminary check, for two months before catching-out began. Although this marking was so intensive that in the end over half the flies in the block were marked, not one of these marked flies was recaptured outside the block.

Regular marking was started in Blocks V and IX at the end of September, 1946. Not one of these marked *G. palpalis* was recaptured for three months, but at the end of December one was taken in Block VII, having crossed from Block V. Thereafter, about two marked immigrants a month were recaptured in Block VII ; they crossed as readily from Block IX (three miles downstream) as from Block V (two miles upstream), the majority being females. At first it seemed impossible that these marked

TABLE VI.

Third phase of Block VII hand-catching experiment.

4 weeks ending	Fly-boy-days	Teneral		Non-Teneral		Grand Total
		Males	Females	Males	Females	
8.1.49	1,084	44	32	98	61	235
5.2.49	1,240	65	51	115	56	287
5.3.49	1,161	36	15	79	45	175
2.4.49	1,005	31	38	118	59	246
Catching continued for a fortnight after 2.4.49 with the following results :—						
	251	11	5	32	14	62
	254	8	14	29	11	62

Note.—As in the case of Table V the above figures include those taken by the land catchers as well as the punt-borne catchers. Of the grand total of 1,067 flies, 987 were taken by the punts.

immigrants represented a number sufficient to have an appreciable effect on the course of the experiment. Towards the end of 1947, however, it became clear that this must be so. In an early attempt to demonstrate the presence of *G. palpalis* in the middle of the clearings, grass screens, as used by Bloss (1945) were built on the river banks. These were at right angles to the river, 50 yards or more long, and ran down to the water's edge (Pl. IV, fig. 3). Catchers stationed at these screens had caught a negligible number of *G. palpalis*. These grass screens were now joined together with a hessian screen, six feet deep, hung from a wire stretched right across the river. One complete screen (consisting of two grass screens with hessian across the river) was put 300 yards from the block from which it was desired to stop emigration ; the second screen was 700 yards further on. Both screens were treated with DDT, and flies were caught off them by a man in a punt. The catchers on the first screen caught about 140 a week, while the one on the second caught about 12 a week. It seemed that flies were successfully passing the screens and this was confirmed by erecting a third screen 700 yards below the second. Some ten flies per week were caught from this screen. The screens had revealed the route by which flies were entering the block, but, even though impregnated with DDT, they were not seriously hindering the flies from crossing the clearing. This is shown by Table V. There is no diminution of the catch after the screens came into operation in January, 1948.

The final attempt to prevent *G. palpalis* from crossing the clearings was by means of " DDT traps ". These consisted either of horizontal cane screens, 2 ft. square, or of wickerwork umbrellas of the same area beneath which pieces of dark blanket were suspended. The " trap " was set just over the edge of the water and treated with DDT once a fortnight. Two hundred traps were placed in each clearing, 100 on each bank, spaced at intervals of 5 yards. Thus, for 500 yards of each isolating clearing, the river was studded on both sides with lethal and seemingly attractive objects, and it was difficult to conceive that *G. palpalis* could pass through without alighting on one of them. Nevertheless, the catches in Block VII showed no diminution compared with previous catches (Table VI). During the period covered by Table VI two non-teneral *G. palpalis* marked in Block V were caught in Block VII. These were put in cages, one survived over 24 hours and one over 48 hours, so that it was unlikely, though not impossible, that either had acquired a lethal dose of DDT in its passage across the clearing.

During the last seven weeks of the period covered by Table VI a number of " foreign " marked male *G. palpalis* were released in Block VII. The recaptures of these marked males were analysed by the method of Jackson (1948). The data are too bulky to include here, but they indicate a very high birth-immigration rate, thus supporting the view that immigration into Block VII was sufficiently heavy to prevent any further progress being made.

It is not known exactly why a size of clearing which has proved effective for over three years on the Sari (a stream drying back to a series of pools) should be quite useless on the permanent Kuja. The two places involved are only six miles apart, and the difference in altitude is negligible. The explanation may well be that the Sari has a V-shaped bed, whereas the Kuja has vertical banks up to 20 feet high. These throw shade for a great part of the day, and it is conceivable that they encourage movement of *G. palpalis.*

Two instances of marked *G. palpalis* travelling exceptionally long distances were noted in the course of this work. In May 1948, one captured in Block V had been marked in Block IX, and in August 1948 one was captured just below Block IX which had been marked in Block V. These flies had each travelled some nine miles following the general course of the river, but the distance they actually flew following the water might have been as much as 13 miles.

To sum up, *G. palpalis* can cross even the lower clearing of 3½ miles. The clearings were " ruthless " and not a single tree was spared (Pl. IV, fig. 1). Moreover, they were maintained meticulously and woody regrowth, or even long grass was not tolerated. Although the number of flies that crossed them was small it was sufficient to make the eradication of *G. palpalis* impossible by hand-catching (or by any other " direct " method which leaves the habitat unaltered). The small number that crossed was extremely difficult to detect and the familiar technique of releasing marked flies on one side of the clearing and searching for them on the other gave completely negative results for five months. It was also extremely difficult to find *G. palpalis* in the clearings.

Summary.

A block of a mile and a half of riverine forest on the Kuja River was separated from other riverine bush by clearings of 2 and 3 miles. Clearing costs are recorded.

An attempt was made to exterminate *G. palpalis* in the block by hand-catching. This failed, because the clearings did not exclude immigrant *G. palpalis.*

More *G. palpalis* could be caught from boats on the river than from paths beside the river.

Two attempts were made to prevent *G. palpalis* from crossing the clearings :—

(a) by stretching hessian screens treated with DDT right across the river.

(b) by placing large numbers of DDT treated " trap-screens " in the clearings.

Neither had a decisive effect.

No evidence was found to indicate that hand-catching would not work in a completely isolated block, but it appears to be quite impracticable to isolate a block of *G. palpalis* bush on a permanent river.

Acknowledgements.

This experiment was carried out under the direction of Mr. Napier Bax, at that time Director of the East African Tsetse Reclamation Department. Mr. W. E. F. Thompson relieved the senior author during his absence on overseas leave. Mr. P. J. Greenway of the E. A. Agriculture and Forest Research Organisation identified our botanical collections. The good work of our African Assistant Mr. Haggai Onono (Pl. IV, fig. 4) has once again greatly eased the burden of day to day supervision. We are indebted to the Air Ministry for permission to reproduce the aerial photograph (Pl. IV, fig. 1) taken by the Directorate of Colonial Surveys.

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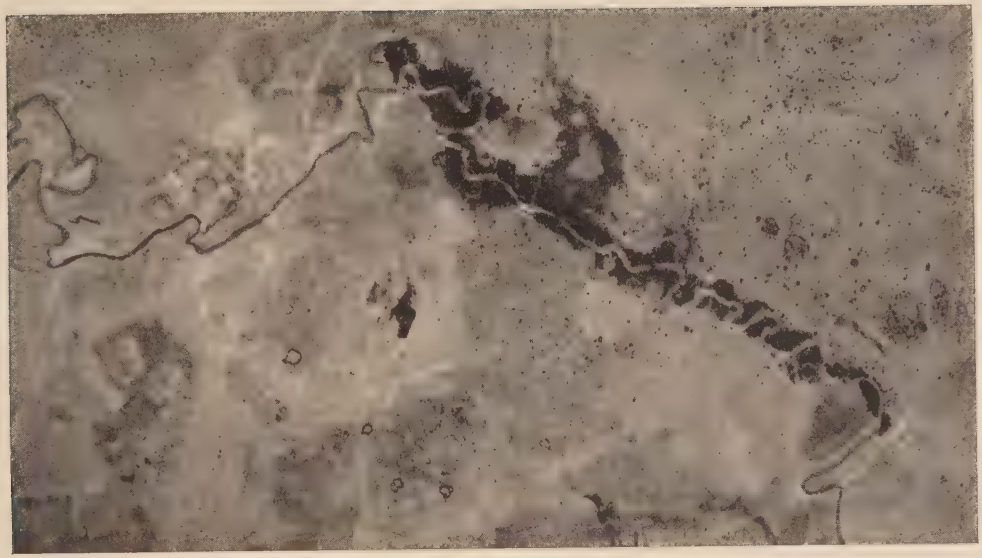

FIG. 1. Aerial photograph of Block VII from 20,000 feet on 20-2-48. Supplied by the Directorate of Colonial Surveys, and reproduced by permission of the Air Ministry.

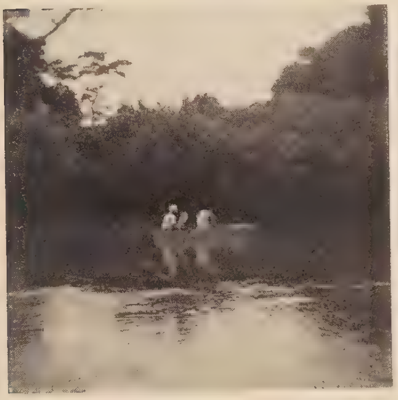

FIG. 2. Punt-borne catchers. *Ficus capreifolia* thicket behind.

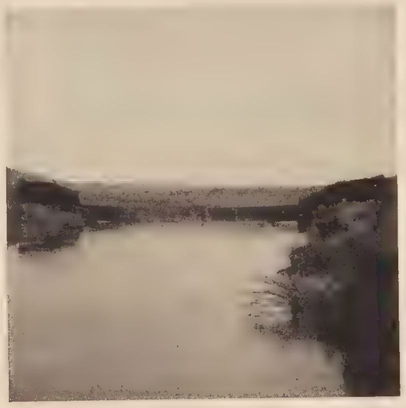

FIG. 3. Grass screens on banks joined by hessian screen across the river.

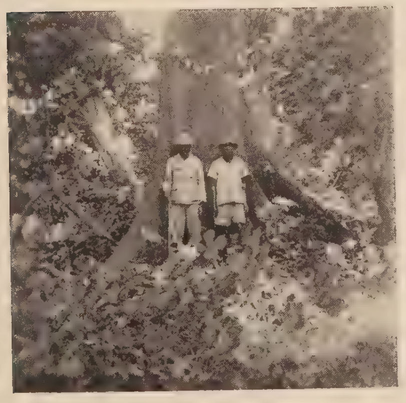

FIG. 4. Trunk of unusually large *Albizzia zygia.*

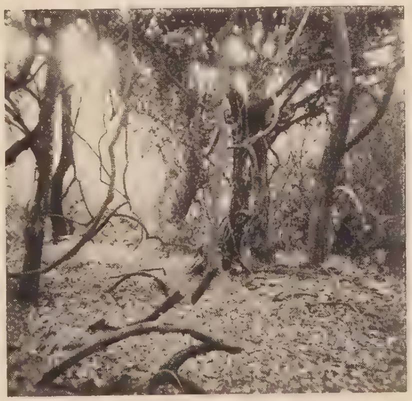

FIG. 5. Trunks of *Pachystela brevipes* with a cut liana in the foreground.

THE ENTOMOLOGY OF SWOLLEN SHOOT OF CACAO.

II.—THE BIONOMICS AND ECOLOGY OF THE SPECIES INVOLVED.

By A. H. Strickland.

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In an earlier paper (Strickland, 1951), those species which were considered to have some bearing on the spread of swollen shoot virus of cacao have been detailed with regard to their taxonomy and biology. In the present paper, the bionomics of these species are discussed in relation to those ecological factors which were considered to have an important bearing on the survival and density of the vectors and their associated insects. The investigations described in the following pages, though covering two years continuous field sampling of the relevant insect species, cannot be regarded as more than an extended series of preliminary observations, and it remains for future workers to elucidate many important points that must be investigated in detail if the ecology of the field transmission of swollen shoot is to be fully understood.

The investigations described in this paper were initiated in May 1947, and at that time very little published work on the problems associated with insect density on tree crops was available for reference. A number of American workers had evolved and published techniques for the estimation of *Citrus* pests (*e.g.*, Osburn & Mathis, 1946 ; Holloway & others, 1942), but only one case could be found of an attempt to assess mealybug populations (Hough & others 1945, *Pseudococcus comstocki* (Kuw.) on apples). Hough's technique consisted of collecting the mealybugs manually on a man-hour basis. Smirnov & Polejaeff (1934) estimated populations of scale insects of the genera *Lepidosaphes* and *Chionaspis* on ash, poplar, and apple trees by taking random bark samples 2 sq. cms. in area, and Dutt (1925) estimated *Monophlebus* density by counting the numbers of bugs caught in sticky bands round the trunks of his experimental trees.

In all of these cases, the insect pests estimated occurred at a density of thousands rather than tens per tree, and it was soon clear that a radically different technique would have to be evolved, based on the nature of cacao as grown in the Gold Coast, and on what was known of the biology of the insects it was desired to estimate. The method eventually adopted is given in detail below.

The purpose of the investigations was, briefly, to discover as much as possible about the ecology of the field transmission of swollen shoot by elucidating the inter-relations of the vectors and their associated insects on a quantitative basis in the field. Unfortunately (but not unexpectedly in view of Broadbent's (1948) results with aphis and potato virus) the hope that a direct association between mealybug density and virus incidence would be established has not been realised. In this respect the investigations have failed in their original purpose. The results obtained are, however, considered to be of wider significance and of more general interest in the field of insect ecology than was originally anticipated, and it is for these reasons that they are presented below in considerable detail.

Methods.

An initial experiment was set up to determine the distribution and density of mealybugs on individual cacao trees. Forty cacao trees were felled at random at a rate of eight per plot on five adjacent one-acre plots, and the mealybugs on them were collected and counted. From the results it was clear that vector density fluctuated widely from tree to tree and plot to plot, that the mean density was of the order of 80 mealybugs per tree, that the preferred feeding sites were the fresh shoots in the canopy, and that nearly 90 per cent. of the total mealybug population was to be found in the canopy, 15 to 20 feet above ground level. Over 80 per cent. of the mealybugs were obscured from sight by ant-constructed carton tents. The experiment further indicated that a sample of the order of 83 per cent. of the total tree population per acre would have to be taken at appropriate intervals in order to determine mealybug density within confidence limits of 10 per cent. at a probability of 0·01. This rate of sampling was clearly not a practicable proposition and it was eventually decided to commence work by felling 12 trees on each of ten one-acre plots per month, a sampling rate which, at the end of the first year's work, was found to give confidence limits of 20 per cent. at a probability of 0·05. It must therefore be understood that, because of the great variability encountered in vector density, only gross differences between plots are shown as significant in the analyses on the succeeding pages.

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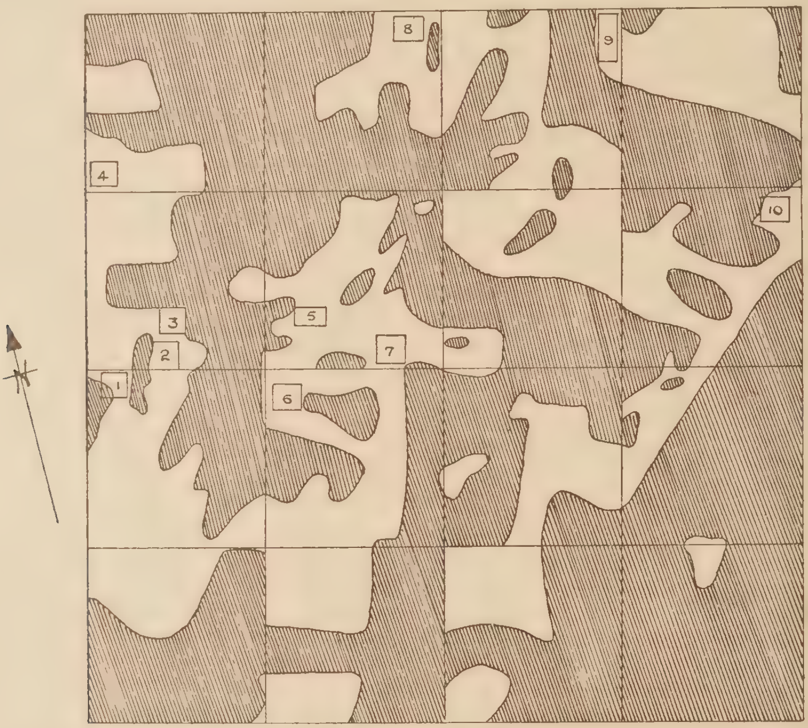

Fig. 1.—Showing the random distribution of the original ten one-acre plots on the 640 acre block of land taken over by the West African Cacao Research Institute in 1945. The shaded areas were not available for experimentation, and the grid was prepared from a detailed map of the unshaded areas.

The West African Cacao Research Institute is situated on a block of 810 acres of Government land at Tafo in the Eastern Province of the Gold Coast. From a detailed map of the cacao remaining on the lands not already reserved for experiments or other purposes a grid was prepared. Each possible acre of cacao, or cacao and small patches of bush, on this grid was given a number. From 350 numbered blocks available, ten were chosen at random by drawing numbers from a hat. The plots were then demarcated in the field as nearly as possible in accordance with the original grid positions. Fig. 1 shows the locations of the ten plots on the 640 acre block of land acquired by the Institute in 1945. The numbered grid was prepared from the unshaded areas.

At the end of the first year's sampling the situation was reviewed and it was decided to change the routine somewhat for the second year. It was desired to continue sampling for a second year on half of the original plots, and to sample five new plots adjacent to the original plot 1 in order to determine the nature of the mealybug distribution on a compact six acre block of cacao.

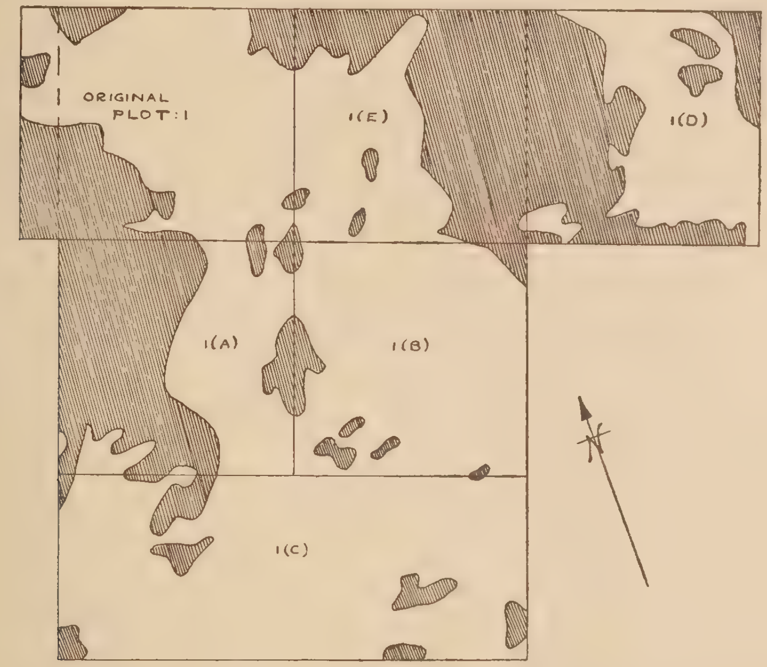

Fig. 2.—Showing the positions of the five new plots set up for the second year's sampling around the original plot 1. The shaded areas denote patches of secondary bush which had sprung up as a result of swollen shoot killing off the cacao which originally covered the whole six-acre block. The block can be taken as reasonably typical of a small cacao farm on the perimeter of the Eastern Province "raging belt" of virus at the present time.

Since plot 1 was to be sampled for a second year, it was decided to continue sampling on all of the odd-numbered plots over the second year. Plot 9, however, had so few trees left on it, and there were so few trees in its immediate vicinity, that plot 8 was included instead. In order to bring the overall tree population for all plots back to roughly 600 per acre, small changes were made in the boundary lines for plots 1, 3, and 7 before the second year's sampling was commenced. Plot 1 was increased in size by roughly 1/16th acre, including an additional 33 trees, plot 3 had included within its boundaries 105 trees growing along the north western boundary line, and plot 7 similarly had a strip added to its north west boundary, including 72 additional trees. The five new plots were set up round the two free sides of plot 1 as shown in fig. 2.

Throughout the sampling period the following routine was adhered to:—On arrival at the appropriate plot the Assistant in charge of the field work entered the plot at one boundary line, walked ten feet, and tied a numbered label on the nearest cacao tree. The other 11 trees in that sample were then selected at random and labelled, a zig-zag course being taken across the plot to the opposite boundary, and one tree being selected every six to eight paces. The Assistant then returned to the first tree. The position of the tree was measured in relation to the two nearest plot boundary lines, and a girth measurement was taken at the one-foot level. The tree was then felled at the one-foot level, and its height was measured to the nearest foot. Any ants present on the trunk or foliage were collected and preserved in spirit in appropriately labelled tubes for mounting and determination in the laboratory. The main and secondary branches were severed and, whilst one assistant was examining the trunk and main branches for coccids, the other members of the gang were severing the leaves, pods, and twigs from the secondary branches and discarding all parts of the tree not infested with coccids. All infested twigs, leaves, etc., were placed in a tin box on the ground, and were in turn carefully examined with a hand lens, and all mealybugs and other coccids counted in relation to their feeding stations (twigs, pods, leaf petioles, etc.). All adult mealybugs were removed and, after three or four had been preserved in spirit for correct determination in the laboratory, the rest were placed in a dry tube for parasite breeding in the laboratory ; all relevant data having been entered on a field record sheet, the gang moved on to the next tree.

At the end of the first year all plots were visited and all trees showing symptoms of virus infection were cut out. Since latently infected cacao showing no visible symptoms can be almost as good a source of virus to the vectors as trees with visible symptoms, this did not interfere seriously with the continued spread of virus within such heavily infected areas as plot 1. In the course of the second year, it became necessary to remove infected trees showing symptoms at the end of each month's sampling ; apart from the fact that this made the plot 1 area appear virus free to a casual observer at any given time, it also probably reduced the total number of infected trees showing symptoms during the second year. The data set out in Table II are thus probably biased, at least as far as the second year's results are concerned.

There were four major disadvantages inherent in the technique outlined above. In the first case, the population estimates obtained were not sufficiently accurate to show up more than gross differences. Secondly, the progressive denudation of the plots was such that it is almost certain that the removal of 25 per cent. of the standing cacao affected the microclimate within the plots and as such may have affected the behaviour of the mealybugs and their ant attendants during the latter part of the first, and throughout the second, year on those plots sampled for two years. However, in an area in which swollen shoot is raging, such progressive denudation over a period of time is in the natural course of events and the monthly felling can be regarded as nothing more than exceptionally severe virus outbreak so far as effect on vector density is concerned. Thirdly, as has already been pointed out, it was not practicable to allow virus to spread naturally on the plots over the two year period. Conclusions with regard to mealybug density and virus spread must therefore be regarded as tentative. Finally, the disadvantage inherent in all insect population work (the removal in each sample of a proportion of the population), the relative importance of which is based on population density and sample size. In the present work it was estimated that, on the average, between 1 per cent. and 2 per cent. of the total mealybugs were removed from a plot with each sample. Such a percentage, though large for most insect census work, was unavoidable in view of the sample size eventually chosen. However, the tremendous variations in density encountered between and within plots, leading to large sampling errors, probably render the artificial decrease in density per month a matter of minor importance.

Ecological Notes on the Plots.

The 15 experimental plots were situated on five different soil series, several of the plots lying on an admixture of more than one series. Mr. C. F. Charter, Chief Soil Scientist, Gold Coast Department of Agriculture, has informed the writer that the important differences between these soil series lie in drainage, and that all of them are developed over biotite, horneblend, and granodiorites. With the exception of plot 8, all plots were on gently undulating land and it is Mr. Charter's opinion that the relatively small differences between series, and the more or less uniform growth of the cacao on the plots, can in no way account for the very great plot differences in mealybug and virus incidence.

Representatives of 56 genera of indigenous forest trees were present on the 15 plots, with a total of 65 individual species. The commonest tree was *Ficus exasperata* Vahl, with 64 out of a total of 463 trees. The Moraceae accounted for 98 trees altogether, the Sterculiaceae 74, Euphorbiaceae 49, and the Apocynaceae 30, trees. Of the Sterculiaceae, *Cola cordifolia* R. Br. was the dominant species with 21 specimens present. This tree has been proved an alternative host of swollen shoot, but there are no indications that its presence has had an effect on the virus incidence in the various plots. Forest tree density varied from 20 to 63 per plot (63 on plot 8, and 52 on plot 1d range otherwise 20 to 35), with a mean of 31 per plot. In general, the Sterculiaceae formed the dominant proportion of the top canopy, the Moraceae being the lower canopy dominants. Members of the Tiliales (Sterculiaceae, Bombacaceae, and Tiliaceae), known or suspected as being alternative hosts of swollen shoot formed together just over 24 per cent. of the total forest tree population.

The distribution of cacao on the plots was not nearly so uniform as the forest trees. At the start of the first year there was a total of 6,095 cacao trees on plots 1 to 10, at the start of the second year 5,951 trees were present on plots 1, 3, 5, 7, 8 and 1a to 1e. The situation is summarised in Table I.

TABLE I.

Total cacao trees on the experimental plots.

Plot	Total trees at start of years sampling	Mean girth (inches)	Mean height (feet)
1(1)*	546	15·6 ± 0·28	22·9 ± 0·31
1(.)*	317	13·5 ± 0·26	20·2 ± 0·42
3(1)	657	13·4 ± 0·38	21·8 ± 0·39
3(2)	611	11·2 ± 0·27	20·2 ± 0·44
5(1)	647	15·2 ± 0·29	23·5 ± 0·34
5(2)	492	13·1 ± 0·28	22·2 ± 0·37
7(1)	752	14·5 ± 0·41	21·9 ± 0·47
7(2)	621	11·0 ± 0·34	19·2 ± 0·47
8(1)	997	10·9 ± 0·32	17·5 ± 0·40
8(2)	853	9·0 ± 0·22	16·9 ± 0·36
2	397	14·7 ± 0·25	22·5 ± 0·38
4	795	14·2 ± 0·41	21·6 ± 0·41
6	527	13·0 ± 0·43	19·0 ± 0·46
9	476	14·2 ± 0·34	20·6 ± 0·52
10	301	16·4 ± 0·29	25·0 ± 0·35
1a	455	14·1 ± 0·30	21·8 ± 0·40
1b	797	12·3 ± 0·27	19·4 ± 0·39
1c	773	12·8 ± 0·29	21·2 ± 0·41
1d	476	11·9 ± 0·27	18·9 ± 0·38
1e	556	13·0 ± 0·30	20·2 ± 0·34

* Throughout this paper the designations (1) and (2) following plot numbers 1, 3, 5, 7 and 8 indicate first and second years' samples respectively.

There is no significant difference between the first and second year means of 610 and 595 trees per plot. There are, however, significant plot differences in girth and height, though height was more uniform than girth. Girth has been shown (Gordon, J., unpublished Report, Gold Coast Department of Agriculture), to be a fair index of age so far as cacao is concerned. Trees of less than eight-inch girth are regarded as immature, and yield is linearly correlated with girth over most of the range. Of all plots, plot 8 was the most divergent, and consisted largely of young cacao just coming into bearing.

Virus incidence differed considerably from plot to plot, and the position is summarised in Table II which shows the total number of virus infected trees (a), felled in the random samples, and (b), cut out at the conclusion of sampling, on all plots, both years.

TABLE II.

Swollen shoot virus on the experimental plots.

Plot	Trees removed with visible virus symptoms	Infected trees felled in random samples	Total virus trees
1(1)	118	19	137
1(2)	123	7	130
3(1)	7	—	7
3(2)	24	1	25
5(1)	11	4	15
5(2)	12	1	13
7(1)	59	15	74
7(2)	158	22	180
8(1)	—	—	—
8(2)	—	1	1
2	1	—	1
4	118	12	130
6	5	5	10
9	2	4	6
10	9	12	21
1a	74	14	88
1b	21	4	25
1c	38	7	45
1d	34	4	38
1e	120	7	127

It is clear that swollen shoot is patchy in distribution. Plot 1, for example, had an abnormally high virus incidence, and so had plots 1e (adjacent to plot 1), and 7. Plot 4 was visited at the end of the second year's sampling and a tree count was made. This indicated that 234 trees had been cut out as infected with virus by the WACRI swollen shoot control gang over the second year, an even greater increase than on plot 7. Plots 2, 8, and 9, on the other hand, appeared virtually free of virus.

Taking plots 1, 3, 5, 7, and 8 for each year separately it is apparent that out of the 3,599 trees present to start with, 233 trees became infected in the first year (6·5 per cent.), and out of 2,894 trees present at the start of the second year, 349 became infected within 12 months, showing an increase from 6·5 per cent. to 12·1 per cent., or a virtual doubling of incidence from one year to the next. These figures are, of course, referable only to trees showing visible symptoms, and take no account of latent infections. Plots 5(1), 6, 9, and 10 appear remarkable on account of the number of infected trees felled in the random samples in relation to the total number of infected trees removed at the end of the first year. Plot 1 (2) is remarkable for the few trees taken in random samples in relation to the number cut out during the second year. In the first instance, it is likely, particularly as far as plots 9 and 10 are concerned, that there were many more trees present with virus than were actually cut out at the end of the first year, symptoms being either latent or obscure and thus missed by the virus control gang. In the case of plot 1(2), infected trees were felled during the second year, each month after sampling, and at any given time it would seem as if there was very little virus on the plot.

It is clear that the spread of swollen shoot can, in certain circumstances, be very rapid. Plot 4, for example, gives a jump from 16 per cent. infection at the end of the first year to over 40 per cent. at the end of the second year. Plot 1 jumps from 25 per cent. to 41 per cent., and plot 7 from 9 per cent. to 29 per cent. over the same period. As such, the natural denudation of an area of cacao by swollen shoot, with attendant effects on the vectors and ants present in that area, is a factor of considerable ecological, as well as economic, importance.

Trees per acre, their age, and the incidence of virus, all have effects on yield in pods per acre. Each plot was harvested six times during the year. This procedure was necessitated because of the high incidence of black pod fungus (*Phytophthora palmivora* Butler). Harvesting was carried out at monthly intervals from September to February inclusive, all ripe pods being removed from each plot once per month. Numbers of mealybug infested pods per plot per harvest were noted, and the mealybugs on these pods counted and identified. Adults were collected, as for the tree felling experiment, and put up in petri dishes for parasite breeding. During the first year only, ant determinations were made of the species attendant on mealybug pod colonies. Table III summarises the harvesting data for the two years.

TABLE III.

Ripe pods per plot and per harvest.

	Plot :—										
Year	1	3	5	7	8	2	4	6	9	10	Totals
1947–48	2,456	3,181	5,467	1,714	802	2,328	4,191	1,146	1,068	3,359	25,712
						1a	1b	1c	1d	1e	
1948–49	965	3,674	5,173	1,417	1,600	3,942	15,353	5,476	3,739	1,926	43,315

	Harvest :—					
	September	October	November	December	January	February
1947–48 ..	6,598	10,453	5,595	1,775	1,166	125
1948–49 ..	17,542	15,809	7,136	2,231	527	70

Harvesting was started late during the second year. 1948–1949 was a very good crop year, and many mature pods were present on the trees earlier in the season than usual. Unfortunately, the writer was on leave in the United Kingdom at this time and was unable to make the necessary alterations in routine which would have been advisable had he known of the early crop in time.

Owing to lack of the necessary recording instruments, it was not possible to obtain detailed meteorological data on the microclimate within the plots. Conditions under the cacao canopy differ markedly from those within a Stephenson screen in an open field, and so the standard measurements of temperature and humidity from the meteorological station at Tafo cannot properly be used as indices with which to compare fluctuations in mealybug density. Rainfall has, however, been selected for analytical purposes.

The Tafo rain gauge is situated between half and one mile from the plots. As, however, there are no great topographical differences on the station lands it was considered that the central rain gauge figures were sufficiently accurate to be used as independent factors in correlation analyses.

When sampling over a period of one month it is clearly not possible to take that month's rainfall as affecting the insect population under investigation. For example, although the rainfall in August 1947 was 7·22 ins., a total of 4·12 ins. fell between

10th and 23rd August and could not have affected the mealybugs taken in the August samples from plots 1, 2, or 3 which were sampled prior to the 10th. Rainfall records have accordingly been split up into groups referable to the various times the individual plots were sampled as follows :—plots 1–5, first year, and 1, 3, 5, 7, and 8, second year, are based on total rainfall from the 1st to the 31st of the month prior to sampling. Plots 6–10 first year, and 1a, 1b, 1c, 1d, and 1e, second year, are based on total rainfall from the 14th of the previous month to the 13th of the sample month inclusive. The sampling year commenced on May 1st and the appropriate figures are given in Table IV.

TABLE IV.

Rainfall at Tafo in inches "per previous sample month."

Sample Number	Plots			
	1–5 1947–48	6–10 1947–48	1, 3, 5, 7 and 8 1948–49	1a to 1e 1948–49
1	7·08	4·58	13·67	16·43
2	6·81	7·94	8·18	3·37
3	8·65	8·83	2·39	1·50
4	4·86	4·67	0·95	2·95
5	7·22	9·17	4·42	6·11
6	12·59	10·15	9·00	6·84
7	4·58	2·58	1·75	2·67
8	3·04	3·28	2·85	0·61
9	0·49	0·02	1·69	3·46
10	0·03	0·32	5·11	7·15
11	5·12	9·70	6·06	4·79
12	7·67	4·63	5·33	5·87

For further details of the climate at Tafo the reader is referred to the Annual Reports of the West African Cacao Research Institute for 1947–48 and 1948–49 (Anon, 1949, 1950).

The Quantitative Results of the Survey.

It has proved convenient to summarise the quantitative results of the field survey in two parts : First, those data concerned directly with the density of the mealybug vectors, and secondly those data concerned directly with the density of the ant species regarded as having an important bearing on mealybug density.

(a) *The Mealybug Vectors.*

In the first paper of this series (Strickland, 1951) it was shown (Table II) that the chief field vector, *Pseudococcus njalensis* Laing, occurred at a density about a hundred times that of *P. citri* (Risso), the next most common cacao mealybug. It was further shown that *P. citri* itself was roughly ten times as common as all of the other cacao feeding pseudococcids together. In view of the overwhelming importance of *P. njalensis* all results in this section (except where otherwise stated), are referable only to this mealybug species.

The total number of trees per sample per plot infested with *P. njalensis* is set out in Table V. From this table it is immediately clear that there are very great differences in infestation rate even between adjacent plots such as 1 and 1a. The first year results from the top half of Table V have been expressed as percentages and transformed according to the Inverse Sine Transformation (Cochran, 1938), and an analysis of variance has been computed. This is summarised below and shows quite clearly that highly significant plot and sample differences are exhibited in the 1947–48 results.

TABLE V.

Trees, per sample, infested with *P. njalensis*.

	Plots :—															
	1	3	5	7	8	2	4	6	9	10	1A	1B	1C	1D	1E	Totals
Sample																
1	9	1	2	3	3	5	1	3	8	1						36
2	9	1	2	7	2	–	–	4	8	1						34
3	9	–	2	4	3	5	2	4	9	2						40
4	12	1	5	4	5	2	–	7	5	4						45
5	9	–	4	5	2	3	2	4	10	4						43
6	8	2	1	2	2	4	3	5	9	1						37
7	7	2	5	5	4	2	2	8	6	4						45
8	12	2	5	3	6	7	1	7	9	4						56
9	8	2	3	7	2	4	3	6	6	7						48
10	12	1	5	6	6	8	1	8	6	6						59
11	10	2	7	5	3	7	2	6	8	7						57
12	11	2	5	6	3	4	3	5	8	7						54
1st yr. Totals	116	16	46	57	41	51	20	67	92	48						554
13	10	2	3	4	3						3	6	5	7	10	53
14	12	3	3	7	–						3	4	3	9	11	55
15	10	1	1	2	5						2	8	4	10	10	53
16	9	3	5	2	2						2	3	4	9	9	48
17	11	1	2	6	1						2	7	6	9	11	56
18	9	–	1	5	4						2	6	7	11	12	57
19	10	1	5	8	3						1	5	5	7	11	56
20	7	3	3	4	2						5	4	4	6	10	48
21	12	1	2	3	2						5	6	9	9	8	57
22	7	2	3	8	3						4	4	5	10	9	55
23	5	3	1	1	3						2	5	4	7	8	39
24	5	2	4	5	–						6	5	3	9	10	49
2nd yr. Totals	107	22	33	55	28						37	63	59	103	119	626

Analysis of variance: Mealybug infestation rate. All plots, 1947–48 only.

Factor	Degrees of Freedom	Mean Square
Between plots	9	2599·24 * * *
Between samples	11	299·54 * * *
Error	99	94·16

In the light of this analysis it was considered worthwhile to investigate the variation within plots in greater detail. It has already been pointed out that the position of each tree was measured at the time of felling. It was thus possible to prepare accurate plot spot plans on graph paper and to divide any plot up into sub-plots of any desired size. Plots 1, 3, and 7 were so treated, both years' samples being included. Each plot was split up into 1/16th acre sub-plots, the trees in the second year plot extensions being ignored, and percentage infestation was computed for each sub-plot on which more than ten trees had been felled. The percentages were converted according to the inverse sine transformation and the three analyses set out below computed :—

Analyses of variance : Mealybug infestation rate. Within plots variation.

Factor	Degrees of Freedom	Mean Square
Plot 1		
Between ¼ acre sub-plots . .	3	328·42
Between 1/16th and within ¼ acre sub-plots . .	7	233·85
Plot 3		
Between ¼ acre sub-plots . .	3	88·21
Between 1/16th and within ¼ acre sub-plots . .	8	156·97
Plot 7		
Between ¼ acre sub-plots . .	3	192·92
Between 1/16th and within ¼ acre sub-plots . .	11	276·50

N.B.—Throughout this paper the following symbols have been used to denote statistical significance :—*** significant at better than p: 0·001 ; ** significant at between p: 0·01 and 0·001 ; * significant between p: 0·05 and 0·01 ; N.S.: Not significant at p : 0·05.

These analyses suggest that, on plots with high, moderate, and relatively low infestation rates there is no more variation in percentage incidence between ¼ acre sub-plots than between 1/16th acre sub-plots within ¼ acre plots. Hence it would not have been possible to obtain substantially more uniform incidence by working with smaller plots.

The total number of *P. njalensis* of all stages of maturity (excluding mature males), collected in the course of the field work is set out on a plot and sample basis in Table VI, the mean infestation rate being 65·7 bugs per tree. In the entire experiment only 26 trees out of a possible 2,880 were felled which supported populations in excess of 1,000 bugs and, of these, five supported populations in excess of 2,000 bugs. One tree in plot 1e supported a population of 3,075 bugs, the highest figure recorded in the quantitative work. In a different experiment, however, in which 20 trees which could be seen to be heavily infested with mealybugs were felled, one tree was found to be supporting a population of 7,371 mealybugs. However, from the main experiment in which all estimates are based on the principle of random sampling, it is safe to say that less than 1 per cent. of the cacao in the Tafo district (roughly in the centre of the main Gold Coast cacao belt), supports populations in excess of 1,000 bugs per tree. Such a density bears very little relation to the "thousands of mealybugs" caught in individual sticky bands by DeBach (1949), on citrus trees in California.

It was considered to be of interest to examine the population frequency distribution on a group of plots forming a continuous area. Plots 1 and 1a to 1e were the most suitable for this purpose, although it is clear that they cannot be regarded as forming a homogeneous area. The distribution found to have the best fit was obtained from the terms of the logarithmic series $\sum \frac{X^{r+1}}{r+1}$ for $X=0{\cdot}9981$, and this is set out in Table VII.

TABLE VI.

Total *P. njalensis* per plot per sample.

	Plots:															
	1	3	5	7	8	2	4	6	9	10	1A	1B	1C	1D	1E	Totals
Sample																
1	2,416	61	27	251	117	751	1	2,597	620	1						6,842
2	5,369	165	532	3,072	1,005	—	—	338	442	26						10,949
3	5,568	—	56	340	472	1,299	118	213	1,196	4						9,266
4	1,947	21	271	758	529	45	—	1,413	441	455						5,880
5	2,708	—	651	395	465	684	146	532	1,868	188						7,637
6	4,552	123	329	190	538	568	92	1,971	870	15						9,248
7	3,644	232	932	436	692	174	197	4,467	1,729	81						12,584
8	4,590	158	441	147	1,441	962	7	580	1,643	350						10,319
9	3,624	479	110	862	189	348	963	238	338	230						7,381
10	5,130	15	980	329	495	566	1	261	1,038	504						9,319
11	3,371	163	355	88	232	555	32	1,335	1,793	491						8,415
12	4,086	18	339	163	100	531	140	1,109	958	1,671						9,115
1st yr. Totals ..	47,005	1,435	5,023	7,031	6,275	6,483	1,697	15,054	12,936	4,016						106,955
13	1,406	56	231	90	66						156	587	178	610	769	4,149
14	2,077	145	106	187	—						162	708	201	1,524	960	6,070
15	1,041	1	11	1,344	347						17	343	328	487	1,848	5,767
16	783	189	336	113	217						194	775	207	195	1,141	4,150
17	364	1	57	496	1,363						91	458	136	724	2,191	5,881
18	1,194	—	23	409	213						130	517	620	2,483	2,713	8,302
19	2,424	62	97	629	1,133						125	340	397	2,183	1,929	9,319
20	734	30	7	229	78						332	478	982	661	1,330	4,861
21	3,546	60	39	67	76						128	166	574	874	3,439	8,969
22	2,169	547	267	129	241						1,040	167	73	331	1,235	6,199
23	2,344	415	3	223	286						29	303	104	395	2,872	6,974
24	1,669	26	122	196	—						302	1,996	54	2,132	5,174	11,671
2nd yr. Totals ..	19,751	1,532	1,299	4,112	4,020						2,706	6,838	3,854	12,599	25,601	82,312

TABLE VII.

P. njalensis population distribution on trees in the group of six adjacent plots, 1 to 1e.

Mealybugs per tree	Observed		Calculated		χ^2
0	396	536	139	526	0·19
1	17		69		
2	16		46		
3	10		34		
4—	97		238		
25—	84		88		0·18
50—	80		76		0·21
100—	69		73		0·22
200—	50		55		0·45
400—	29		32		0·28
800—	16		14		0·29
Total ..	864		864		N.S. 1·82

It can be seen from Table VII that the fit of the logarithmic series is very bad indeed in the smaller classes 0 to 25. This may well be an indication of faulty field work. If a good part of a tree has been examined and no mealybugs found, then it seems likely that less care would be taken in the remainder of the search than if some bugs had already been found. Conversely, the discovery of one bug would tend to make the search more careful. On one occasion the writer asked to see the field sheet for the penultimate tree felled when supervising the field work on plot 2. Apparently there were no mealybugs on this tree. The writer then examined the remains of the tree himself, and within five minutes had found three adult and two nymphal *P. njalensis* hidden in a crack in the bark of one of the secondary branches! It seems likely, then, that the lower terms of the observed frequency distribution are not as reliable as the higher terms.

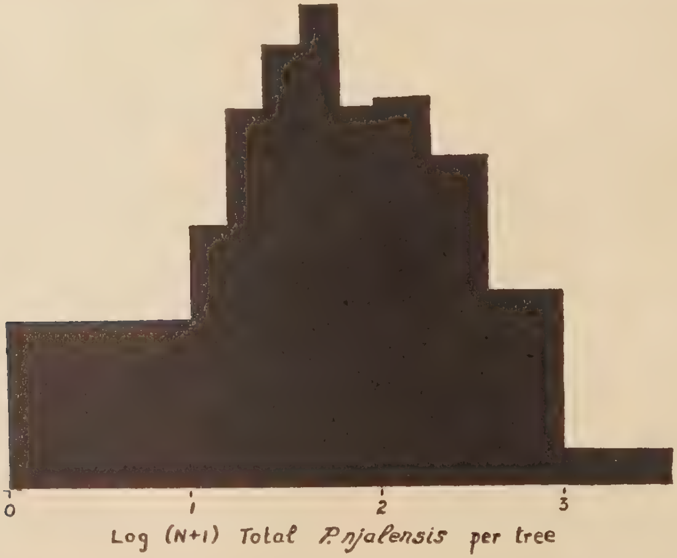

Fig. 3.—Histogram compounded of data from all plots, both years, showing the almost normal frequency distribution obtained by transforming the individual mealybugs per tree figures according to Williams' Log. (N + 1) transformation.

Tables VI and VII indicate that the distribution of *P. njalensis* is markedly skew and therefore some sort of transformation is necessary before the field data can be subjected to statistical analysis. After due consideration, it was decided to use the Log. (N + 1) transformation as described by Williams (1937). Individual tree totals were converted to Log. (N + 1), and the transformation, summed to sample totals, is given in Table VIII. The histogram shown in fig. 3 has been prepared from a frequency table for all plots, both years, and shows that the log. transformed data give a distribution which is much closer to the normal than that given by the untransformed field results. It should, however, be noted that trees with no mealybugs have been omitted from the histogram, and that the large number of these inevitably introduces considerable skewness even into the transformed data.

Two analyses of variance have been computed from the Log. (N + 1) transformed data for total *P. njalensis*. The first analysis, set out below, was computed from data from all plots, first year only, and confirms the conclusion from the tree infestation analysis given on a preceding page. Between plot differences are highly significant, and there is a significant plots/samples interaction indicating that the individual plot populations differed in their reactions to ecological factors operating ever a period of 12 months. It is possible, of course, that such factors as insolation had a greater effect on some plots than on others, and that the interaction is an indication of this rather than an indication that the mealybugs themselves differed in their reactions to factors operating at a uniform intensity on the different plots. The between samples mean square is not significant when the variance ratio is computed from the interaction mean square, showing that there are no consistent differences in population between months.

Analyses of variance.—Total *P. njalensis*, all plots, first year only, and total *P. njalensis*, plots 1, 3, 5, 7 and 8, both years. Log. (N + 1) transformed data.

Factor	Degrees of freedom	Mean Square
(1) All plots, first year only.		
Between plots	9	35·59 * * *
Between samples	11	1·31 N.S.
Interaction	99	2·74 * * *
Error	1,320	0·60
(2) Plots 1, 3, 5, 7 and 8, both years.		
Between plots	4	90·58 * * *
Between years	1	12·39 N.S.
Between months	11	0·84
Interactions :—		
Plots × years	4	2·81 * * *
Plots × months	44	0·54 N.S.
Months × years	11	0·84 N.S.
Plots × months × years ..	44	0·75 N.S.
Error	1,320	0·65

The second analysis was based on data from the five plots samples for the entire two-year period. Plot differences are again highly significant and there is in addition a significant plots by years interaction indicating different long term population trends on the individual plots. There is again no indication in the second analysis of a significant month to month variation, nor of any consistent difference between the years for all plots.

The conclusions that can reasonably be drawn from the data presented on the preceding pages are summarised as follows :—

Firstly, there is great variability in *P. njalensis* population density and infestation rate even within relatively small areas of uniform mature cacao.

TABLE VIII.

P. njalensis tree counts transformed to log. (N + 1) and summed to sample totals.

						Plots :									
	1	3	5	7	8	2	4	6	9	10	1A	1B	1C	1D	1E	Totals
Sample																
1	18·93	1·79	2·00	4·34	4·57	9·34	0·30	6·27	11·02	0·30						58·86
2	22·31	2·22	4·79	13·39	5·38	—	—	6·49	12·09	1·43						68·10
3	22·32	—	2·56	6·34	4·32	9·96	2·37	6·67	17·60	0·90						73·04
4	23·48	1·34	8·29	8·71	8·41	2·73	—	14·62	8·59	6·50						82·67
5	20·77	—	7·94	8·66	3·15	6·29	3·05	6·19	19·82	6·12						81·99
6	19·02	2·68	2·52	3·89	4·80	7·57	4·31	10·18	16·42	1·20						72·59
7	15·74	3·75	10·00	6·10	7·59	3·08	3·67	14·78	13·48	4·13						82·32
8	29·63	2·68	8·05	4·55	10·25	12·87	0·90	9·99	16·48	7·27						102·67
9	19·43	3·82	3·68	11·40	3·71	7·32	5·80	7·91	8·46	7·71						79·24
10	28·87	1·20	7·98	7·86	9·75	10·98	0·30	9·71	10·34	9·75						96·74
11	21·94	2·69	8·60	5·65	4·98	10·88	2·16	11·84	16·02	8·83						93·59
12	24·59	1·88	6·99	7·68	4·57	7·48	4·58	9·51	12·07	9·01						88·36
1st yr. Totals ..	267·03	24·05	73·40	88·57	71·48	88·50	27·44	114·16	162·39	63·15						980·17
13	17·47	2·33	5·26	4·77	4·00						5·09	8·42	7·62	13·09	17·36	85·41
14	23·88	5·03	3·73	9·02	—						5·03	7·73	3·20	16·95	17·72	92·29
15	16·79	0·30	1·08	4·74	7·76						1·85	9·19	4·50	15·18	19·73	81·12
16	15·89	4·36	8·48	3·41	3·62						3·72	6·20	5·68	10·08	18·08	80·26
17	13·57	0·30	2·06	10·08	3·13						2·43	11·26	7·07	14·50	21·33	85·73
18	16·03	—	1·38	7·60	5·03						3·51	9·30	12·48	21·02	25·32	101·67
19	20·65	1·80	4·98	11·20	6·81						2·10	6·05	7·66	13·17	20·22	94·64
20	12·74	2·06	1·48	5·76	3·19						6·98	6·48	7·93	10·30	18·86	75·78
21	22·86	1·79	2·49	3·76	2·83						5·51	7·00	13·08	15·61	18·79	93·72
22	15·07	3·04	5·29	8·74	5·40						7·14	5·78	4·53	13·16	15·64	83·79
23	11·88	4·38	0·60	2·35	4·69						2·29	7·53	4·61	9·40	17·57	65·30
24	8·65	2·17	5·80	7·42	—						9·37	10·95	3·05	18·91	18·54	84·86
2nd yr. Totals ..	195·48	27·56	42·63	78·85	46·46						55·02	95·89	81·41	172·11	229·16	1024·57

Secondly, there is evidence to indicate that those ecological factors which affect *P. njalensis* density do not operate consistently in time over even small areas of cacao. For example, the mealybugs on plots 1 and 3 differed markedly in their reactions to factors operating within an area of ten acres over a two year period, in spite of the fact that the cacao host plants on the two plots were of uniform age and growth.

Thirdly, there is no evidence of significant major variations in density occurring from month to month over a two-year period, though it is admitted that the sampling technique adopted was probably not sufficiently refined to show up minor seasonal variations.

The pertinent results relating to the other cacao pseudococcids are summarised in Table IX. It is clear that the between plots variation in infestation rate and density is of the same order as for *P. njalensis*. Since the populations are so small, no attempt has been made to analyse the individual plot results statistically.

TABLE IX.

Totals of other pseudococcid species, all plots, both years.

Plot	Trees infested with		Total mealybugs collected	
	P. citri	*All other species*	*P. citri*	*All other species*
1(1) ..	31	12	118	54
1(2) ..	31	3	443	4
3(1) ..	10	6	50	11
3(2) ..	9	2	15	2
5(1) ..	8	2	22	10
5(2) ..	11	2	14	2
7(1) ..	20	4	37	51
7(2) ..	5	2	8	5
8(1) ..	17	7	121	10
8(2) ..	7	2	36	2
2 ..	18	10	145	19
4 ..	12	3	26	9
6 ..	29	7	136	10
9 ..	19	5	29	5
10 ..	14	5	224	5
1a ..	12	5	83	6
1b ..	9	3	51	3
1c ..	13	1	49	1
1d ..	14	5	159	5
1e ..	23	4	173	5
Totals ..	312	90	1,939	219

From a comparison of the plot totals for trees infested with *P. njalensis* (Table V), and the first column of Table IX it would seem as if areas with a high *P. njalensis* infestation rate also tend to have a relatively high *P. citri* infestation rate. A correlation analysis computed from the Table V totals and the first column of Table IX gives a coefficient of 0·702 with 19 degrees of freedom indicating that there is a close association between the two species. It would seem, therefore, that certain areas are more suitable than others for the survival of both species. A correlation computed from the individual sample totals in Tables V and VIII gives a coefficient of 0·945 with 239 degrees of freedom showing that there is an extremely close association between *P. njalensis* infestation rate and overall density of the bugs. It would seem reasonable to suggest that the same is the case for *P. citri* and the other species but unfortunately the data are too few to give a reliable indication.

TABLE X.

Ant associations on Cacao.

	Mealybug attending genera					Non-Mealybug attending genera			
	Crematogaster								
	(Sphaerocrema)	*(Atopogyne)*	*(Crematogaster)*	*Pheidole*	*Atopomyrmex*	*Macromischoides*	*Oecophylla*	*Polyrhachis*	*Camponotus*
Times collected in whole experiment	760	251	269	114	20	313	952	207	50
Camponotus ..	8	19	2	5	—	20	4	17	4
Polyrhachis ..	57	36	33	20	1	57	71	33	
Oecophylla ..	150	17	168	40	2	31	511		
Macromischoides	12	64	13	13	1	152			
Atopomyrmex ..	16	1	—	—	-				
Pheidole	13	12	12	41					
(Crematogaster) ..	14	—	69						
(Atopogyne) ..	3	134							
(Sphaerocrema) ..	521								

(b) *The Ant Species.*

The fact that certain ant species tend to be closely associated with various homopterous insects has been known for many years but has received little detailed study. It was known at the time the field survey was being planned that *P. njalensis* was commonly associated with ants of the genera *Crematogaster* and *Pheidole*, and the survey was accordingly extended to include quantitative data on all ant species occurring on cacao which might be expected to exert some influence on the presence and density of the mealybug vectors.

There were two major problems to be considered ; First, the degree, and nature, of interspecific competition between the ant species inhabiting the cacao canopy and, secondly, the nature of the association between certain of the ant species and the mealybugs and the effect of this association on mealybug density.

The three dominant arboreal ant genera *Crematogaster*, *Oecophylla* and *Macromischoides*, have been selected for detailed consideration, the data appropriate to the other genera being inadequate for analytical treatment.

An indication of the degree of competition existing amongst a variety of the arboreal genera is given in Table X. The top line of this table shows the total number of times each genus was collected in the whole experiment. The body of the table shows the number of times each genus was collected as the only ant present on a felled tree in relation to the number of times that genus was collected from trees also infested with the other genera named. There is a marked difference between the total number of trees infested with the individual genera and the number infested with these genera alone. *Oecophylla*, for example, was collected from 952 trees, on 441 of the occasions being taken along with one or more other ant species from the same tree.

TABLE XI.

Mean log. (N + 1) Coccids per tree when ant species occur alone and together on the same tree.

	Mean *P. njalensis* per tree	Mean *Stictococcus sjöstedti* per tree
With *Sphaerocrema* ants only	1·703 ± 0·017	0·013 ± 0·009
With *Oecophylla* only ..	0·107 ± 0·066	1·757 ± 0·058
With both ants together	1·602 ± 0·109	1·332 ± 0·129

Perhaps the most noteworthy fact shown in Table X is that only occasionally is more than one type of true mealybug tending ant found on a single tree. There are exceptions to this. *Atopomyrmex*, for example, is an avid mealybug tender and on 16 out of the 20 times it was collected it was present with *Sphaerocrema* ants on trees infested with *P. njalensis*. On the other hand, it is quite clear that a greater proportion of the total *Atopogyne* ants were found on trees devoid of other *Crematogasters* and *Oecophylla*. This is not surprising because *Atopogyne* ants sporadically attend both mealybugs and Stictococcid scale insects and are thus liable to be drawn into active competition between both ant groups. There are also indications in the Table that *Oecophylla* can exist on the same tree as *Sphaerocrema* or *Crematogaster* ants. Since *Oecophylla* is an avid Stictococcid tender and ignores mealybugs, and the two Crematogasterine genera are avid mealybug tenders and ignore Stictococcids, it is reasonable to postulate that so long as the Coccids are few in number, and are present on different parts of the tree, then each ant is content with its own territory, and that the two groups will only tend to be drawn into active competition as a result of over-population by the Coccids. That this latter condition may occur is indicated by Table XI from which it is clear that bigger mean populations of the two Coccid species tend to occur when only one ant species is present than when both ant species are present. The table is compounded from the five plots sampled for both years.

A further indication of the degree of interspecific competition existing between the three dominant ant groups is afforded by the data set out in Table XII. This table shows the overall plot totals of trees infested with the respective ant groups in relation to the numbers of trees per plot on which the groups were taken together. A series of two-way tables has been set up and chi-squares computed for *Crematogaster/Oecophylla* and *Crematogaster/Macromischoides* trees. All associations were found to be negative, the *Crematogaster/Oecophylla* results being mostly highly significant. The extremes of frequency encountered in the *Crematogaster/Macromischoides* tables necessitated the use of Yates' correction for continuity, and a number of plots had to be omitted altogether because of inadequate data. In addition, overall correlation coefficients for the three groups have been computed for all plots and samples. These results do show consistent negative correlations between the various genera—though the correlation between *Oecophylla* and *Macromischoides* is non-significant. The most outstanding negative association is certainly between *Crematogaster* and *Oecophylla* and may well, as pointed out above, be due to the respective coccidophilic habits of these two genera. Since *Macromischoides* is definitely not a coccidophilic species it would seem as if there is little reason why it should trespass into *Crematogaster* or *Oecophylla* territory and if this is the case then it is to be expected that negative associations would be less pronounced than between the other genera.

Table XII also indicates the degree of between plot variation in density encountered amongst the three ant genera. Analyses of variance computed on all plots, first year, showed that between plot differences were highly significant for all three genera, but that seasonal differences were non significant.

In the whole experiment 2,230 out of the 2,880 trees felled were infested with one or more of the many ant species involved. Yet on only 169 trees (less than 8 per cent.), were three or more ant species present on the same tree, and on 30 trees only were four or more species found. Five species were present on three trees, and six species were present on only one tree. In the latter case *Macromischoides*, two species of *Polyrhachis* and one each of *Atopogyne*, *Camponotus* and *Platythyrea* were present. Of the three trees with five species all were infested with two species of *Polyrhachis* and one of *Atopogyne*, and two of them were also infested with *Pheidole*. It is clear that, on occasion, individual cacao trees do support a variety of ant species but it cannot be denied that such occasions are rare and that more often than not a single species, or not more than two species occupying distinct ecological niches, are present.

The Factors that influence the Density of the Mealybug Vectors on their Cacao Host.

From the accumulated field data it has been possible to evaluate the effects of a number of ecological factors on mealybug density and these are discussed under appropiate sub-section headings below.

(a) *Food Supply.*

The cacao mealybugs* occur at such a low mean density that it seems reasonable to suggest that competition for available food supplies is not an important factor in natural control. There are no indications from the field data that mealybug density is in any way associated with overall density of the cacao—in fact a correlation

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* In this section, as in the last, all results are referable to *P. njalensis* only unless specified to the contrary

TABLE XII.

Total sampled trees per plot with certain ant genera alone and together.

Plot	*Crematogaster* trees	*Oecophylla* trees	*Macromischoides* trees	*Crematogaster* and *Oecophylla*		*Crematogaster* and *Macromischoides*		*Oecophylla* and *Macromischoides*	Trees with all three genera
				trees	χ^2	trees	χ^2		
1	226	59	0	21	80·58***	0	—	0	0
2	46	50	20	22	5·12*	3	1·33N.S.	0	0
3	71	58	75	3	14·84***	20	0·01N.S.	2	0
4	25	66	26	10	0·41N.S.	5	—	4	1
5	114	89	22	28	3·55N.S.	5	2·12N.S.	1	0
6	58	63	13	21	2·25N.S.	4	0·19N.S.	1	0
7	106	167	6	42	23·22***	0	—	1	0
8	86	50	44	8	5·55**	6	5·65**	1	1
9	70	70	12	25	9·07***	4	0·65N.S.	4	2
10	42	93	19	19	9·70***	5	0·30N.S.	6	1
1a	65	51	31	14	9·98***	14	—	3	1
1b	64	54	9	17	5·88**	2	—	0	0
1c	68	55	19	19	5·74**	10	0·07N.S.	6	3
1d	123	9	16	4	12·94***	11	2·65N.S.	1	0
1e	123	10	1	6	44·80***	1	—	0	0
	Overall correlation coefficient with 239 df :—			—0·329***		—0·314***		—0·085N.S.	

between the cacao trees per plot figures in Table I and the total log. (N + 1) mealybugs per plot figures in Table VIII gave a coefficient of 0·07 which is not significant. Neither are there any indications that mealybug density is associated with mean yield of pods per tree or overall yield of pods per plot. Harvesting of ripe pods does, however, have an effect on mealybug density. Of the 25,712 ripe pods harvested from all plots during 1947–48, 1,542 were infested with *P. njalensis*, 84,013 mealybugs being counted. These results give an overall mean of 54 mealybugs per infested pod, or 3·3 per pod.

At the start of the 1947–48 harvest there were 5,495 cacao trees still standing on plots 1–10. This number was decreased by 120 per month so that by the sixth harvest in February 1948, only 4,895 trees were still standing on the plots. With a mean estimate of 5,200 trees over the harvesting period, and 25,712 ripe pods collected, the yield worked out at five pods per tree. If each ripe pod was infested by roughly 3·3 mealybugs, then 16·5 bugs per tree were removed over the harvesting period. With a mean density of 65·7 bugs per tree these results would seem to indicate an annual loss of the order of 25 per cent. of the total *P. njalensis* population over the harvesting period. Ripe pods are undoubtedly an attractive feeding site for *P. njalensis* and this is indicated by an estimate of the percentage of infested pods re- and the infestation rate rose steadily to 8·0 per cent. in November and 15·2 per cent. in February. At the same time, mean infestation rate rose from 2·2 at the start of the season to 5·4 bugs per pod. There is, then, a pronounced establishment of new mealybug colonies on pods as the pods develop and reach maturity and since most mealybugs on ripe pods are destroyed in the harvesting, breaking, and husk dumping processes the formation of pod colonies becomes an important factor in natural control of population density on cacao.

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It was considered that growth and age of the host cacao might affect the density of the mealybugs. Frequency tables were prepared for all plots, both years, showing trees per girth and height class, numbers infested with *P. njalensis*, and numbers of *P. njalensis* per class. The data are summarised in Table XIII.

Omitting the first and last classes in each case owing to lack of adequate data, correlation coefficients have been computed on mean class frequencies (6, 10, 14, etc.), and the percentage of infested trees per class. For girth, the coefficient was 0·959, and for height 0·861, the girth coefficient being significant at p. = 0·001, and the height coefficient at 0·02. There is therefore some evidence that the longer a cacao tree has been standing the more likely it is to be infested with mealybugs. Taking girth as a more accurate estimate of age than height, there are also indications that trees of more than 18-inch girth tend to support considerably higher mealybug populations than younger trees of smaller girth. It should perhaps be emphasised that an old tree of large girth with a relatively high mealybug population may support either a population which has resulted from a single initial infestation and has over a period of years increased in size, or a population built up of several successive infestations over a period of time. In either case, it would appear that the older the tree the more likely it is to become infected with swollen shoot—particularly if the theory of multiple infestation is correct. Unfortunately the dispersion of the 139 trees with swollen shoot (felled in the field samples), amongst the girth classes was such as to render the computation of a correlation coefficient impracticable so quantitative evidence to support the assertion cannot be produced at present.

A third factor in food supply which it is appropriate to consider in this section is virus incidence. As has been pointed out in the first paper, when a mealybug infested cacao tree is felled, dies, and desiccates, the mealybugs migrate, or get carried, off it. The same takes place in the case of dead, but still standing, swollen shoot trees, a tree killed by virus never having been seen infested with mealybugs by the writer. Indeed, in areas where virus has already taken heavy toll of the standing cacao, mealybug populations appear to be well below normal. Anon. (1948), gives

84. Insert after line 18 "moved per harvest. In September, 3·1 per cent. of the total ripe pods were infested"

TABLE XIII.

P. njalensis infestation in relation to girth and height of host cacao.

GIRTH					HEIGHT				
Class (inches)	Number of trees		% trees infested	Mean *P. njalensis* per tree	Class (feet)	Number of trees*		% trees infested	Mean *P. njalensis* per tree
	Infested	Not infested				Infested	Not infested		
0— 3·9	—	3	—	—	0— 4	1	—	100	23
— 7·9	65	201	25	35	— 8	10	25	29	29
—11·9	258	500	34	44	—12	73	123	37	73
—15·9	467	585	44	70	—16	158	226	41	70
—19·9	303	323	48	91	—20	233	336	41	75
—23·9	69	77	47	94	—24	374	525	42	57
—27·9	13	8	62	99	—28	266	394	40	65
—31·9	3	2	60	64	—32	59	57	51	81
—35·9	2	1	67	5	—36	—	—	—	—

* Height measurements for 20 out of the 2,880 trees felled were not taken owing to an error on the part of the chief field assistant.

results from 150 trees felled in a heavily infected virus outbreak. In this survey 23 per cent. of the trees were infested with mealybugs, with a mean population of 17 bugs per tree. Again, plot 1, heavily infested with mealybugs in May 1947, supported a very much smaller mean population in 1949 when virus and tree felling had taken a heavy toll of the standing trees.

The two years' field data do not, however, give any indication of a close correlation between vector density and virus incidence, and this fact is seen clearly from Table XIV which summarises the virus/vector situation on five representative groups of plots.

TABLE XIV.

Virus incidence and mean log. (N + 1) Mealybug density per tree on a representative group of plots.

	Trees felled without virus		Trees felled with virus	
	Number	Mean *P. njalensis*	Number	Mean *P. njalensis*
1, both years, and 1e second year	399	1·591	33	1·722
1a, b, and c, second year ..	407	0·546	25	0·402
7, both years	251	0·605	37	0·419
4, first year	132	0·175	12	0·365
10, first year	132	0·453	12	0·278

Mealybug populations were higher on infected trees in only two out of the five groups. Even in the contiguous area plots 1 and 1a to 1e there is no consistent indication of either positive or negative associations between mealybugs and virus.

There are several possible reasons for this anomalous state of affairs. In the first place, it is almost impossible to obtain accurate data on rate and direction of virus spread from field plots selected strictly at random. The virus incidence on plot 4, for example, was undoubtedly in part due to the felling of a large number of infected mealybug-infested trees adjacent to the north eastern boundary in 1946. Again, it was not possible to tell definitely whether the virus in plot 1 was spreading into plot 1e, or *vice versa*.

Secondly, where there is no virus there cannot be any spread however many mealybugs there may be. Thirdly, as has already been pointed out, areas very heavily infected with virus tend to have a lower mealybug population than areas of healthy cacao or areas in which virus can be said to be of fairly recent introduction and in which the cacao canopy is still largely intact.

The only reasonable conclusions that can be drawn from the available data, then, are that initial virus incidence can in certain circumstances (*e.g.* plot 4), be extremely high with low populations of vectors and that, in areas that have been devastated by virus, vector populations are likely to be low. Hence there can be little doubt that swollen shoot, by destroying a large proportion of the favoured plant hosts, is an important factor in natural control of the mealybug vectors.

(b) *Climate.*

As has already been pointed out, rainfall is the only climatic factor that has been considered in relation to mealybug density.

The annual rainfall at Tafo over the last ten years has varied from 49 to 69 inches per annum, with a mean of 61·6 inches. There is one well marked dry season in December to February, when the rainfall drops to roughly 2·4 inches per month, and a less well marked dry period in July–August, with a fall of roughly 2·6 inches per month. The climate is hot and humid, and in marked contrast to those relatively dry areas in Kenya and California where mealybugs are pests in their own right and probably reach densities of hundreds of thousands per host plant.

Individual plot and year correlation coefficients have been computed using the monthly rainfall figures shown in Table IV and total log. (N + 1) mealybugs per sample for total bugs and for several distinct parts of the total population. The results are summarised in Table XV, and show that rainfall has little effect on mealybug density. In fact, of the seven significant coefficients, three are positive and four are negative! Most of the individual plot coefficients are, however, negative and all but one of the overall coefficients are negative, an indication that the effect of rainfall is more likely to be one tending to a decrease, rather than an increase, in density of *P. njalensis*.

It is certainly the case that some species of mealybugs which breed up to pest proportions during the dry season are very rare during the wet season (Strickland, 1947), and though the field data do not show that this is the case with *P. njalensis* it is undoubtedly true to say that torrential rain does have an effect on the density of most of the cacao mealybugs. It is thus of some interest that the overall coefficient for all other pseudococcids (–0·146 with 239 degrees of freedom), is significant at between p. = 0·05 and 0·02.

(c) *Ants.*

Out of the 189,267 *P. njalensis* counted in the field survey, 185,945 were directly associated with Crematogasterine or Pheidoline ants of known coccidophilic habit,* and only 840 mealybugs were collected from trees on which no coccidophilic ants could be found. The ratio of 840 to 189,267 is approximately 1 in 225, and this compares with a *P. citri* ratio of 1 in 6 (317 unattended to 1,939 collected), and a ratio for all other species of 1 in 2·3 (93 unattended to 219 collected). It is apparent, then, that *P. njalensis* is almost invariably associated with ants in the field and that the several mealybug species under consideration vary considerably in their " attractiveness " to the various attendant ant species.

In an attempt to determine whether attendant ants were essential for the very existence of *P. njalensis*, an experiment was set up under controlled conditions in an insectary. Atmospheric humidity was maintained by artificial watering at between 90 per cent. and 100 per cent. and temperature fluctuated between 27°C. (at 02·00 hrs.), and 39°C. (at 15·00 hrs. G.M.T.) daily. Twenty individual six-month old cacao seedlings were each infested with four young adult females of *P. njalensis* collected from naturally infested cacao in the field. Weekly counts were made on the 20 colonies so established. After 26 weeks four generations had been completed, the mean infestation rate being 429 ± 127 bugs per plant, indicating quite conclusively that *P. njalensis* can maintain itself perfectly well when completely isolated from ant attendants. After the same period the control colonies, attended by *Crematogaster africana* (Mayr), were infested with a mean of 156 ± 52 bugs. In this experiment the unattended mealybugs rapidly became covered with mould growth (*Penicillium* spp. with an occasional *Aspergillus*), which grew readily on the unremoved sugary secretions exuded by the bugs. The moulds did not, however, seem to interfere seriously with the capacity of the bugs to reproduce and thrive. Several of the cacao plants used also became thickly covered with moulds, without any apparent deleterious effects. Indications are, then, that the moulds are purely saprophytic, and the only effect noticed so far as the mealybugs were concerned, was a tendency to form large

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* A total of 121,651 *P. njalensis* were directly associated with the *Sphaerocrema* group of ants.

TABLE XV.

Correlation coefficients, mealybugs and rainfall per month.

Plot	Total *P. njalensis*	*P. njalensis* not protected by ant tents	*P. njalensis* on canopy shoots not protected by ant tents	*P. njalensis* crawlers only	*P. njalensis* crawlers not protected by ant tents	All other cacao Pseudococcids bulked
1(1)	—0·384	—0·215	—0·387	—0·277	+0·029	—0·491
1(2)	—0·002	+0·661*	+0·474	—0·126	—0·484	—0·359
3(1)	—0·275	—0·213	—0·213	—0·369	+0·126	—0·075
3(2)	+0·057	+0·303	+0·042	—0·163	+0·018	+0·170
5(1)	—0·421	—0·275	—0·270	—0·464	—0·162	—0·660*
5(2)	—0·022	+0·475	+0·454	—0·016	—0·151	—0·001
7(1)	—0·181	—0·315	—0·302	—0·079	—0·040	—0·038
7(2)	+0·034	+0·034	—0·361	—0·260	—0·057	—0·281
8(1)	—0·503	—0·441	—0·249	—0·453	—0·012	—0·059
8(2)	—0·210	+0·093	+0·093	—0·446	—0·059	+0·063
2	—0·077	—0·431	—0·422	—0·080	—0·231	—0·490
4	+0·151	—0·154	+0·319	—0·060	+0·072	—0·136
6	—0·217	—0·329	—0·292	—0·202	—0·124	—0·522
9	+0·702*	+0·200	+0·297	+0·675*	+0·458	+0·426
10	—0·448	—0·587*	—0·598*	—0·347	—0·463	—0·224
1a	+0·149	+0·454	+0·454	+0·203	+0·153	—0·008
1b	+0·254	+0·288	+0·368	—0·009	—0·174	—0·237
1c	+0·081	—0·194	—0·143	—0·150	—0·143	—0·474
1d	+0·098	—0·214	—0·214	—0·136	—0·160	—0·077
1e	—0·113	—0·014	—0·013	—0·353	—0·001	—0·264
Overall Correlation	—0·030	—0·020	—0·021	—0·011	+0·012	—0·146*

numbers of small colonies on a plant rather than to remain as one or two compact colonies as on the control, ant attended, plants. This was obviously a " swamping " reaction, induced by the fact that the adult females were so smothered in moulds that the young crawlers had to migrate to a clean part of the plant in order to find a reasonably attractive feeding site.

It has been noted above that the " attractiveness " of individual mealybug species seems to vary considerably as far as the attendant ant species are concerned. Where an obviously attractive species evolves, as in the case of *P. njalensis*, it is rapidly taken under the care and protection primarily of the *Sphaerocrema* group, and to a lesser extent of the other groups, whereas the less attractive species are not cultivated to nearly the same extent. From this it would seem reasonable to suggest that the attractiveness of a given mealybug species to a given ant species is not a simple tactile response. In the field, *P. citri* and *P. njalensis* are extremely hard to distinguish. The external appearance and body texture of the two species are almost identical. Probably, then, the relationship is chemical rather than tactile, and it must be assumed, pending further work, that it is the chemical constituents of the secretions that attract ants so readily to *P. njalensis*. Added weight is lent to this hypothesis by that fact that the secretions of the other Pseudococcid species do not become infected with saprophytic moulds to nearly the same extent as *P. njalensis* when not ant attended, leading to the conclusion that there may be a fungicidal substance or, perhaps more likely, an absence of certain compounds essential to fungal growth, in the secretions of the other mealybug species.

So far as *P. njalensis* and the *Sphaerocrema* group are concerned, it would seem that the obligatory nature of the association is an ant rather than a mealybug factor. Out of 760 trees in the whole experiment with this ant group on them, 701 were also infested with *P. njalensis*. Of the balance of 59 trees, 12 were infested with *P. citri* (Risso), *P. longispinus* (Targ.), or *P. bukobensis* (Laing), ten were infested with *Stictococcus* spp, and four with other Coccid species, leaving 33 trees out of the 760 (4·3 per cent.), on which the field staff were unable to find any Coccids. This compares with 86 trees out of 1,180, or 7·3 per cent. infested with *P. njalensis* apparently without attendant ant species.

This association between *P. njalensis* and the local ants could not be maintained unless it was of considerable benefit to both the ants and the mealybugs. In fact, in exchange for a copious supply of sugary secretions, the ants build carton tents over the mealybugs and thus protect them from external environmental factors.

Wheeler (1906), in his paper on *Crematogaster lineolata* Say, gives four probable reasons for the tent building habit. Firstly, for the purpose of preventing the escape of the Aphids or Coccids inside to other plants, or to other parts of the same plant ; secondly, for the purpose of protecting these insects from their natural enemies or from other ants ; thirdly, for the purpose of protecting them from the cold, and fourthly, for the purpose of protecting themselves from exposure. As evidence to support the first point Wheeler cites instances in which the ants rapidly carry their mealybug cattle to a place of safety when the nests are disturbed, and concludes :— " It is probable that the aphids and coccids make the same appeal to the ants' sense of ownership as their own larvae and pupae." The habit of carrying off the mealybugs to a safe place is well marked in all of the local mealybug tending Crematogasterini. Where, however (as noted in the first paper), the ant's nest is destroyed *Crematogaster africana* will first try to re-establish itself before its mealybugs. In this species at least the coccidophilic habit takes second priority to survival of the queen and brood.

The main effect exerted by the ants on mean density of *P. njalensis* is undoubtedly protective, and a product of the tent building habit of the species concerned. It is of some interest in this respect that the entrances to Crematogasterine tents are usually just large enough to allow the ants to enter and leave. They are too small to allow adult mealybugs to leave, or to allow large ants such as *Oecophylla*, or adult Coccinellid beetles, to enter.

On the 1,180 *P. njalensis* infested trees examined in the whole experiment there was a total of 23,082 mealybug colonies, of which 18,730 were protected by ant tents, and 4,352 untented. Of the total of 189,267 *P. njalensis* taken, 167,842 (88·7 per cent.) were obtained from the tented colonies, the balance of 21,425 being unprotected. From these figures it is apparent that the overall mean colony size of *P. njalensis* is of the order of 8 bugs. The largest tented colony so far examined by the writer consisted of 3,758 bugs, on a cacao pod. The term ‘ colony ” is here used to describe an aggregate of one or more mealybugs, feeding in close proximity, but distinctly isolated from any other mealybugs present on the tree.

The tree felling data, though giving good estimates based on random sampling, do not cover several important aspects of tent protection. A separate experiment was thus set up in which 150 carton tents built over mealybug colonies infesting twigs and shoots, and 150 tents over pod colonies of mealybugs, were measured, and then broken open and the mealybugs inside counted. Tent selection was non-random, only those tents within easy reach of the ground being examined, and anything from one to ten tents being examined on any given tree. The results are summarised in Table XVI and show quite conclusively that pod tents are significantly larger in area than twig tents (not surprising because there is usually a larger area of surface per given length of pod than in the same length of twig or shoot), and that in both cases there are highly significant correlations between tent size (as measured by surface area in square inches), and log. (N + 1) numbers of mealybugs inhabiting them.

TABLE XVI.

Surface area covered by carton tents, and mean log. (N + 1) numbers of mealybugs inhabiting them.

Tent location	Mean area, sq. ins.	Mean log. (N + 1) mealybugs	Correlation coefficient
Shoots and twigs ..	0·22 ± 0·04	1·140 ± 0·039	0·334***
Pods	0·78 ± 0·13	1·107 ± 0·067	0·408***

The largest twig tent seen was 3·0 x 1·5 ins., and contained 79 mealybugs ; the largest pod tent was 4·5 x 2·3 ins., and contained 1,156 mealybugs. The tent containing 3,758 bugs previously referred to was circular, of 1·0 in. diameter. Only one twig tent contained more than 500 bugs (actually, 571), whereas four pod tents contained more than 1,000 bugs, and three others contained over 500 bugs.

As might be expected, the number of tents varied from plot to plot. In order to determine whether there were any seasonal changes in numbers of tents built by the attendant ants the individual ‘ tents per tree ” counts were transformed to log. (N + 1), and summed to plot and sample totals. The two analyses of variance computed—all plots, first year, and plots 1, 3, 5, 7 and 8 both years— showed no significant differences so far as samples were concerned but highly significant differences in numbers of tents per plot. The second analysis also showed a significant (p.=0·001), plots/years interaction, and a similarly significant fall in tent numbers between the two years.

It might be expected that tent numbers would be related to Crematogasterine ant density and an overall correlation has been computed on these two factors. A coefficient of 0·599 was obtained indicating a highly significant degree of association between ant and tent density. A second overall correlation coefficient was computed to indicate the degree of association between Crematogasterine ant density and mean numbers of *P. njalensis* per ant tent per sample per plot. In this analysis a coefficient of 0·157, significant at a probability of 0·02, was obtained indicating that ant incidence does have an effect on mean mealybug density inside tents.

These correlations were computed from data referable to all Crematogasterine ants bulked. Taking those trees on which *Sphaerocrema*, *Crematogaster* and *Atopogyne* were respectively the only mealybug tending ant groups present, mean log. (N + 1) numbers of tents per ant tree were computed. The results showed that trees with *Sphaerocrema* ants had a log. (N + 1) mean of 1·013 ± 0·020 tents, trees with *Crematogaster* ants had 0·592 ± 0·034 tents, and *Atopogyne* trees had 0·262 ± 0·028 tents. The tent building habit, then, may be better developed in certain Crematogasterine ants than in others. These results also may be an indication of a more advanced coccidophilic habit in those species which are inveterate tent builders. Since it is difficult to understand why tents should be built for no apparent reason, it would seem that the habit is dependent on coccidophilism rather than *vice versa.*

It is reasonable, in the light of the results enumerated above, to suggest that Crematogasterine ant density affects the number of ant tents in a given area, the numbers of mealybugs inside the tents, and the size of the tents.

In view of the obvious importance of the attendant ants in relation to the survival of *P. njalensis* in the field a number of analyses have been computed to provide an estimate of the degree of protection provided by the ants. In most cases, these analyses are based on unequal numbers in the different classes and the technique advocated by Yates (1934) has been employed.

The first two analyses are based on the mean log. (N + 1) populations of *P. njalensis* per tree set out in Table XVII. Results from the plots sampled continuously over both years have been bulked in this table in order to obtain adequate numbers of variates on which to base the means.

TABLE XVII.

Mean log. (N + 1) *P. njalensis* per tree in relation to attendance by various ant groups.

Plot	Trees with attendant ant species	Trees with no attendant ant species	Trees with *Sphaerocrema* ants	Trees with any other attendant ant species
1	1·978	0·066	2·049	1·009
3	0·459	0·026	1·551	0·176
5	0·734	0·067	1·764	0·419
7	1·249	0·111	1·683	1·045
8	0·941	0·024	1·468	0·369
Overall mean ..	1·072 ± 0·025	0·059 ± 0·027	1·703 ± 0·017	0·604 ± 0·016

The analyses of variance computed from the data summarised in Table XVII are set out below. The very marked reduction in the between plots mean square compared with the mean square shown in the overall analysis on page 77 is a good indication that at least part of the difference between plot populations can be accounted for by the differing extent to which the various ant groups are present on them. In the second analysis the highly significant difference between ant groups indicates conclusively that the *Sphaerocrema* group is associated with consistently higher mealybug populations than all the other groups.

Analyses of variance, ant groups and *P. njalensis* density.

Factor	Degrees of freedom	Mean square	Variance ratio
(1) *Attendant ants vs. no ants*			
Between plots	4	19·30	1·14 N.S.
Between ant groups	1	321·50	19·04 ***
Interaction, plots × ants ..	4	16·89	40·37 ***
Within groups	1,430	0·42	
(2) *Sphaerocrema ants vs. all other attendant species.*			
Between plots	4	6·37	3·05 N.S.
Between ant groups	1	127·35	60·94 ***
Interaction, plots × ants ..	4	2·09	3·74 ***
Within groups	709	0·56	

In view of the great importance which must be attributed to the Crematogasterine ants as protectors of the mealybug vectors it was considered advisable to investigate the matter in greater detail. Comparisons have thus been made between the mean *P. njalensis* populations when the mealybugs were attended by the various groups of ants only. Unfortunately, the number of trees on which Pheidoline ants were the only ants present was too small in relation to the Crematogasterine groups for any valid comparisons to be made. The three important Crematogasterine subgenera, *Sphaerocrema*, *Atopogyne*, and *Crematogaster*, have therefore been selected for comparison. Three analyses were computed but were not all based on data from the same plots, the plots being selected as having a sufficient number of trees with each ant group on them. The *Sphaerocrema/Crematogaster* analysis was based on data from plots 2, 3, 5, 7, 9, 1b, and 1c ; *Sphaerocrema/Atopogyne* was based on plots 3, 5, 7, 1c, and 1d ; and *Crematogaster/Atopogyne* was based on plots 3, 5, 7, 10, 1a, and 1c. It is of some interest that plots 1 and 1e do not appear in any of the analyses, this being due to the fact that there were less than ten trees on these plots infested with either *Crematogaster* or *Atopogyne* alone. Both of these plots were in many ways exceptional in so far as *Sphaerocrema* was virtually the only ant group present. The analyses based on the means and differences summarised in Table XVIII can therefore be regarded as giving a good estimate of the differences in mealybug density when under attendance by the various ant groups within one acre blocks in which none of the ant groups was overwhelmingly dominant.

TABLE XVIII.

Mean log. (N + 1) *P. njalensis* per tree when attended only by the ant groups specified.*

Ant group	Mean density per tree	Difference between groups
Sphaerocrema	1·682 ± 0·196	0·633 ± 0·275
Crematogaster	1·049 ± 0·193	
Sphaerocrema	1·604 ± 0·197	1·053 ± 0·283
Atopogyne	0·551 ± 0·203	
Crematogaster	0·897 ± 0·204	0·353 ± 0·275
Atopogyne	0·544 ± 0·184	

* Means based on data from plots detailed in the text above.

Sphaerocrema is clearly the most important ant group and is consistently associated with higher mealybug populations than either *Crematogaster* or *Atopogyne*. There are indications that *Crematogaster* tends to be associated with higher mealybug densities than *Atopogyne* though this may well be due to chance.

In view of the very considerable decrease in the between plots mean square noted in the " *Sphaerocrema* vs. all other attendant ants " analysis, it was considered worthwhile to compute overall between plots mean squares for total mealybugs on the plots selected for the generic comparison analyses. The results are set out in Table XIX, and show quite clearly that the mean squares are considerably smaller when those trees infested by more than one mealybug attending ant species, or not infested by any such species, are omitted from the plot analyses. Once again it is clear that mealybug populations are much more uniform from plot to plot when under attendance by Crematogasterine ants.

TABLE XIX.

Comparison of between plot mean squares for total trees and for ant group trees.

Between plot mean squares for :—	*Sphaerocrema* vs. *Crematogaster*	*Sphaerocrema* vs. *Atopogyne*	*Crematogaster* vs. *Atopogyne*
All trees on plots ..	16·03	26·08	27·51
Trees with ants only ..	4·60	3·82	5·48

From the evidence presented in the preceding pages one important conclusion may be drawn. This is that the density of *P. njalensis* in a given area of cacao is to a great extent dependent on the nature and density of the ant groups present in that area.

(d) *Parasitism.*

The technique used for estimating percentage parasitism in the present work was not adapted to give a valid estimate of attack by predatory insects such as beetle larvae and ectoparasitic Cecidomyiids which may feed on more than one host insect in the course of their life cycles. The results presented in this section are thus referable to Hymenoptera only.

Parasite determinations were made from part only of the first year's collections, 442 Hymenoptera being submitted for determination out of the 1,215 specimens collected. Table XX, therefore, gives a rough indication only of parasite dominance.

TABLE XX.

Parasites identified in first year's samples.

Parasite	Plot :— 1	2	3	4	5	6	7	8	9	10	Totals
Anagyrus spp. ..	232	20	2	3	5	21	3	12	24	2	324
Neodiscodes martinii ..	3	4	—	—	6	5	5	3	3	7	36
Tropidophryne melvillei ..	—	2	—	—	—	—	—	—	—	—	2
Cheiloneurus carinatus ..	—	—	—	—	—	—	—	—	1	4	5
Undetermined Encyrtidae ..	23	1	3	8	1	19	—	9	8	3	75

The group of *Anagyrus* spp. consists largely of *A. pullus* Compere the other (probably three), species involved occurring only in very small numbers. It is clear that *Anagyrus* is the dominant parasite genus so far as *P. njalensis* is concerned, and that it appears even in areas such as plot 4 where the mealybug population is very small.

In the whole experiment, plot harvesting both years included, a total of 66,901 *P. njalensis* were put up for parasite breeding, and a total of 1,826 Hymenoptera emerged, an overall parasitism of 2·73 per cent. (293 Cecidomyiid predators were obtained at the same time but for obvious reasons the figure cannot be used to compute percentage attack.) Percentage hymenopterous parasitism per plot from the tree felling data only is summarised in Table XXI.

TABLE XXI.

Percentage hymenopterous parasitism of *P. njalensis*.

Plot	Total *P. njalensis* put up	Total Hymenoptera emerged	Percentage parasitism
1(1)	14,162	756	5·34
1(2)	4,185	214	5·11
3(1)	536	16	2·99
3(2)	187	4	2·14
5(1)	1,750	43	2·46
5(2)	150	2	1·33
7(1)	1,276	37	2·90
7(2)	437	9	2·06
8(1)	1,583	54	3·41
8(2)	494	7	1·42
2	2,251	82	3·64
4	591	11	1·86
6	5,284	96	1·82
9	4,899	82	1·67
10	1,643	38	2·31
1(a)	463	43	9·29
1(b)	993	14	1·41
1(c)	533	13	2·44
1(d)	2,042	60	2·94
1(e)	3,828	146	3·81
Totals	47,287	1,727	3·65

In view of the great importance to be attached to the attendant ants, the parasitism of the colonies under attendance by the most important coccidophilic ant groups has been computed and is set out in Table XXII. All cases in which less than 25 mealybugs were put up for breeding, and all cases in which more than one species of coccidophilic ant were present on a given tree, have been excluded from this analysis. So, in effect, the analysis consists of a comparison of the parasitism rates of *P. njalensis* under attendance by the various ant groups only. The individual tree parasitism percentages have been converted according to the inverse sine transformation. Since there were differing numbers of variates in each ant group it is not possible to compute a single error variance for all groups.

Table XXII indicates that *Sphaerocrema* ants are associated with a significantly higher parasitism percentage than any other group apart from *Atopogyne* though this may well be due to the very small numbers of variates available for computation in the other groups. The other four groups together give a bulked mean of 6·21 ± 0·82 based on 88 variates. This is significantly smaller than the *Sphaerocrema* mean of 9·24 ± 0·44, and there is thus some justification for saying that *Sphaerocrema* is really associated with a higher percentage of parasitism of *P. njalensis* than the other groups.

TABLE XXII.

P. njalensis parasitism under attendance by various ant groups.

Ant group	Transformed Data. Mean	De-transformed. Means (%)	Number of Variates
Sphaerocrema	9·24 ± 0·44	2·6	315
Atopogyne	7·66 ± 2·50	1·8	15
Pheidole	6·99 ± 1·93	1·5	20
Crematogaster	5·51 ± 1·06	0·9	45
No ants at all	5·46 ± 1·94	0·9	8

The 315 variates from which the *Sphaerocrema* mean was computed were obtained from all plots except 1a, and 126 of the variates were referable to plot 1 : Plots 1e and 1d ascribed 45 and 27 variates respectively, the other plots all ascribing less than 25 variates.

Owing to the paucity of variates the available plots have been divided into two groups—those with mean *P. njalensis* populations greater than, and those with mean populations less than, 80 per tree. Plots 1, 6, 9, 1d, and 1e fall in the first group and all other plots in the second group.

TABLE XXIII.

P. njalensis parasitism when under attendance by *Sphaerocrema* ants only.

Mean mealybug density per tree	Transformed Data Mean	Number of Variates
Plots with more than 80 bugs per tree ..	10·26 ± 0·50	231
Plots with less than 80 bugs per tree ..	6·42 ± 0·80	84

There are indications in Table XXIII that percentage parasitism is higher on plots which are heavily infested with mealybugs, a result which is in general accord with accepted theory.

Although the parasitism data considered in this section are not numerous, one valid conclusion can be drawn. This is that normally hymenopterous parasitism of *P. njalensis* in the field is extremely low. Whether this is a reflection of the extremely low normal density of the bugs, or whether it is partly due to the assiduous attentions paid by ants in driving away the natural mealybug parasites, is unknown.

(e) *Incidence of other Insects.*

A certain amount of quantitative data were collected on the incidence of several minor pests of cacao, and on the cacao Mirids, in order to see whether mealybug density was in any way dependent on, for example, changed ecological conditions as a result of severe attack by insects which themselves were not concerned in the transmission of swollen shoot. The two Coccid species, *Stictococcus sjöstedti* Ckll., and *Ceroplastes* sp. nr. *zonatus* Newst., were counted individually in the same way as *P. njalensis*. Trees infested with the cacao aphis, *Toxoptera coffeae* (Nietner), and the cacao Psyllid, *Mesohomotoma tessmanni* (Aulm.), were noted, and damage by the cacao Mirids, *Distantiella theobroma* (Dist.), and *Sahlbergella singularis* Hagl., and the cacao thrips, *Selenothrips rubrocinctus* (Giard), was recorded as " severe " when more than 30 per cent. of the tree showed signs of severe attack, or " slight " when little or no damage could be seen. It should be noted that those trees recorded as severely damaged by Mirids had actually been attacked some time prior to sampling and the bark of the main and secondary branches was in most cases badly scarred and cankered. As such, it was observed that old Mirid lesions provided good feeding stations for *P. njalensis*, the mealybugs crawling deep into cracks and crevices in the bark and receiving good protection from inclement climatic conditions as a result. So far as thrips damage was concerned, it was considered that the defoliation resulting from severe attack might well have an effect on the canopy microclimate and render the cacao less suitable for the survival of the mealybugs.

The pertinent results in respect of *Stictococcus* have already been summarised in Table XI, and it is clear that the scale insect and the mealybug are in no way positively associated. A correlation carried out on trees per plot infested with *Stictococcus* and trees infested with *P. njalensis* gave a coefficient of –0·181 with 19 degrees of freedom which, though not significant, indicates a negative association. A similar correlation between *Ceroplastes* and the mealybug gave a coefficient of 0·836, showing that *Ceroplastes* tends to occur on trees infested with *P. njalensis*, though from observation this is certainly an ant association, *Ceroplastes* being eagerly tended by *Sphaerocrema* ants.

A summary of the relevant results relating to the other insects named above is given in Table XXIV, and it should be noted that the Mirid and Psyllid results are referable to all plots, first year only.

TABLE XXIV.

Mean *P. njalensis* populations on trees with and without certain other insect pests of cacao.

Trees with	Mean Log. (N + 1) *P. njalensis*	Difference
Severe Mirid damage	0·779 ± 0·047	0·163 ± 0·056 * *
Slight or no damage	0·616 ± 0·030	
Severe Thrips damage	0·680 ± 0·041	0·011 ± 0·051 N.S.
Slight or no damage	0·691 ± 0·030	
Both severe Thrips and Mirid damage	0·763 ± 0·062	0·097 ± 0·067 N.S.
Not severely damaged by both pests together	0·666 ± 0·026	
Psyllids	0·790 ± 0·071	0·172 ± 0·074 *
Psyllids absent	0·618 ± 0·021	
Aphids	0·594 ± 0·075	0·044 ± 0·078 N.S.
Aphids absent	0·638 ± 0·021	

It is evident from these results that trees which have been severely attacked by Mirids do tend to support more mealybugs than undamaged trees. It would hardly be reasonable, however, to attribute this state of affairs to a direct association between the two types of insect and pending further work on the problem it must be assumed that the overall effect of Mirid attack, with the associated canker-forming fungus, *Calonectria rigidiuscula* (Berk. & Brm.) Sacc. (Crowdy, 1947), is to render trees more suitable for mealybug colonisation in view of the plentiful protected feeding stations produced by bark splitting and cankering.

The significant Psyllid result is difficult to explain. Of the 215 psyllid-infested trees felled, only 97 (45·1 per cent.) were infested with *P. njalensis*, so it cannot be said that there is a close association between the two species. In fact, a correlation computed on trees per plot with the two species gave a coefficient of 0·011, with 19 degrees of freedom, which is not significant. There is apparently no relationship between severe thrips attack and mealybug density.

Discussion of the Survey Results.

Before discussing the field survey results in detail it seems advisable to set down such scanty information as is available on the normal rate of increase of *P. njalensis*.

It has been pointed out on a previous page that four adults per plant increased to 430 over a six month period. These results indicated that one adult female would give rise to roughly 107 females in four generations, an increase of just under five times per generation. In a second experiment set up under the same conditions in the same insectary each of 16 cacao seedlings was initially infested with one mature adult. The mean population after four generations was found to be 135 ± 33 bugs per seedling. These results indicated a mean increase per generation of just over five times under ant, parasite, and predator free conditions, with an abundant food supply and complete protection from the physical effects of rainfall and direct sunlight. With a fivefold increase per generation and eight generations per annum, theoretically one adult female should give rise to more than 78,000 female progeny over a period of one year on cacao as a host plant. That this is not the case under natural conditions in the field is immediately apparent from Table VI.

The theory of Balance of Animal Populations (see Nicholson (1933) ; Nicholson & Bailey (1935) ; Varley (1947) ; Solomon (1949), and the literature cited by these authors) attempts to explain the maintenance of a more or less steady population density around which the numbers of a species may fluctuate widely but which must, on the average, be maintained if the species is not to become extinct. Briefly, a steady density is maintained through control factors operating less severely on individuals when the density is low and more severely during periods of abundance. A species is unable to increase in density beyond a certain limit imposed by a complex of factors expressed as the " Resistance of the Environment ". Two groups of factors defined respectively as density dependent and density independent are commonly regarded as primarily responsible for the maintenance of steady density though, as Nicholson points out, the " properties of the species " (presumably the innate properties), also play a part. What, then, are the factors responsible for the natural control of population balance in *P. njalensis* at a level very much below that which can be maintained under controlled conditions in the laboratory ?

It has been clearly demonstrated (Tables XVII and XVIII) that mealybug density expressed in terms of " mean mealybugs per tree " is high when certain attendant ants are present and extremely low when no ants are present. (This phenomenon is further clarified in Table XXV, the column "Complete Protection " having been added as an indication of the density which *P. njalensis* can reach on a tree the area of feeding space of which is roughly comparable to that on a mature cacao tree. The balance of the table has been compounded from the results from the five plots sampled for two years.) Since it would be unjustifiable to assume that the same ecological factors act with identical intensity against the survival of the mealybugs whatever habitat they occupy (reference the conclusions drawn from the analyses of variance on page 77), it is proposed to discuss the control factors appropriate to the three cacao niches specified in Table XXV.

P. njalensis is ovoviviparous and of low fecundity compared with other members of the genus. Under controlled conditions in an insectary it has been demonstrated that the normal rate of increase is of the order of five times per generation. So, for a steady density to be maintained this fivefold increase must be balanced by a mortality of 80 per cent. of those bugs that succeed in surviving to the adult stage. Or, taking the mean fecundity of *P. njalensis* to be roughly 36 then 35/36ths of the progeny of each female must fail to produce living progeny or must succumb to environmental factors before the egg laying stage is reached.

Harvesting is a factor which will act independently of ants. It has been pointed out on page 84 that about 25 per cent. of the total mealybug population is destroyed annually in the harvesting process, so that the cacao farmer himself is an important factor in control.

TABLE XXV.

Mean log. (N + 1) density of *P. njalensis* per tree when inhabiting four different ecological niches.

	Complete protection (domatia in *Canthium*)	Obligatory ant protection (cacao and *Sphaerocrema* ants only)	Sporadic ant protection (cacao, all other mealybug tending species)	Completely unprotected (cacao, no attendant ants)
Mean log. (N + 1) density per tree	3·952 ± 0·031	1·703 ± 0·017	0·604 ± 0·016	0·059 ± 0·027
Maximum and minimum fluctuations in mean density per tree per month recorded in the two-year period.	?	2·428 ± 0·113 1·249 ± 0·312	0·916 ± 0·256 0·245 ± 0·197	0·165 ± 0·079 0·009 ± 0·0088

Swollen shoot virus in itself can also be regarded as an important controlling factor. In the first paper of this series (Strickland, 1951—Table IV), indications are given that cacao is the preferred host of *P. njalensis* though it is admitted that much higher densities are reached on *Canthium.* It is sufficient to say that cacao is the most important and most abundant food plant of *P. njalensis* in the Eastern Province of the Gold Coast, and accordingly any factor acting against the survival of the cacao is bound to affect the density of the mealybugs provided, as is the case, that there is an inadequacy of desirable alternative food plants. The effect of swollen shoot is, from the available evidence, independent of mealybug density in so far as a large proportion of the available hosts can be killed with a prevailing low density of vectors (*e.g.* plot 4). Swollen shoot is, in fact, indiscriminate in its effects on the density of any insect species of which cacao is the primary food plant.

Since the transmission of swollen shoot is proof of the fact that the vectors must have fed on both an infected and a healthy cacao tree it is proof also of vector migration and since mature adult mealybugs tend to remain static it follows that tree to tree migration is probably mainly nymphal in character and further, when a tree becomes an unsatisfactory host for the vectors as a result of virus, then it is more likely to be the nymphs which will migrate to a more satisfactory host and the mature adults which will tend to remain on the original host. The progeny of adults left on a dying tree will find it increasingly difficult to survive owing to an unsatisfactory supply of food, increased exposure to climatic factors induced by defoliation and die back, and the necessity of migrating to a fresh host over the ground litter in view of the broken canopy. So swollen shoot can also be regarded as a factor operating directly against adult mealybugs through their sluggish habit, and as a delayed factor against the nymphal stages, though it is freely admitted that this is an over simplification of the natural state of affairs.

To a limited extent, climate can be said to act as a controlling factor regardless of ant protection. Table XV indicates that the effect of rainfall on *P. njalensis* is more likely to be one tending to a decrease than an increase in density, and excess of rain in an abnormal wet season will tend to increase the severity of attack by Black Pod Disease, hence reducing the number of pods available for colonisation by the mealybugs.

It is difficult to see how the factors enumerated above could effect control over some 97 per cent. of the total population of *P. njalensis*, or 80 per cent. of the adult females. Table XXV indicates quite clearly that population density, when the mealybugs are under the care and protection of *Sphaerocrema* ants, is many times higher than when no ants are present, and it would therefore seem reasonable to assume that the ants are directly responsible for protecting their cattle from a variety of important controlling factors.

Nearly 80 per cent. of the mealybugs collected in the field survey were protected by carton ant tents, and tent protection can be regarded as of benefit to the mealybugs in two ways. First, tents protect the bugs from the direct physical effects of rainfall and insolation, and secondly they protect the mealybugs from direct attack by many large predatory insects. Rainfall has been shown to be relatively unimportant as a direct factor in the control of *P. njalensis* even when that part of the population not protected by ant tents is considered, and this may well be an indication of the large number of other desirable feeding stations on a mature cacao tree, the branches of which may be severely cankered as a result of Mirid attack, and the trunk of which is often smothered with lichens. So although *P. njalensis* exhibits a well marked negative phototropism it seems unlikely that insolation is an important factor in control. It would therefore seem that the most important function fulfilled by the tents is one of protection against predatory enemies, and it is notable in this respect that the entrances to Crematogasterine tents are invariably too small to allow large predatory insects to enter.

However, the fact remains that 80 per cent. of the adult female mealybugs inhabiting tents must fail to produce living progeny if the steady density of the species is to be maintained, and since the mature females are sluggish by nature it would seem as if the necessary mortality factors must operate within the confines of the tents. The number of possible factors operating in this way is limited, and in the present case would appear to be restricted to overcrowding phenomena, leading to migration of nymphs out of the tents, and direct predation and parasitism of the remaining adults.

Certain of the attendant ant species (in particular *Crematogaster africana*), are known to be predacious on young mealybug larvae, but the most important tent predators appear to be a group of Cecidomyiid midges, the larvae of which are actively predacious on all stages of the mealybugs. So far as the hymenopterous parasites are concerned, the local species rarely parasitise other than adult mealybugs. Oviposition by *Anagyrus* spp. takes place shortly after the mealybugs have emerged from the third instar and larval development takes place inside the body of the host, completion to emergence taking roughly 14 days under field conditions. Observations have shown that only one parasite develops to maturity in a single mealybug and several mealybugs may be parasitised by one female *Anagyrus*. The highest percentage of parasitism (based on 25 or more host mealybugs put up), encountered in the whole experiment was on a tree in plot 1, in which 31 out of a possible 46 adults were put up and nine *Anagyrus* emerged—a percentage of 29. On the whole, however, the degree of hymenopterous parasitisation is low. This does not mean that it is necessarily unimportant. The success of *Anagyrus* as parasites locally appears to be in their ability to counter the attacks of attendant ants. Once a parasite has entered a tent and successfully parasitised some of the young adult mealybugs residing therein it seems likely that the second parasite generation on emergence will be conditioned, in the same way as the mealybugs, to the ant nest odour and may therefore tend to escape attack. If this is the case, then, on emergence there would be within the confines of the tent a population of young adult mealybugs at the right stage for parasitisation and defenceless against attack, and the parasitism rate would rise temporarily until the majority of the adult mealybugs emerging inside the tent were killed. Such a reaction may take place but it is obvious that it could not take place unless, immediately after the parasites emerge, there is a ready supply of new hosts available. Owing to the extended oviposition period of *P. njalensis*, with resultant overlapping of generations in any given colony at any given time the fact remains that there is almost invariably an adequate supply of young adult mealybugs available for parasitisation. It is clear that a great deal more work will have to be done on the problem before the degree of importance of *Anagyrus* spp. as a controlling factor can be assessed.

It has been pointed out on page 97 that a species is unable to increase in density beyond a certain limit imposed by a complex of factors expressed as the Resistance of the Environment. The field census results show quite conclusively that *P. njalensis* is cushioned against many environmental factors primarily by *Sphaerocrema* ants, and that the mean density of the species is much higher than would be the case if no ants were present. It seems extremely likely, then, that the existing density level is " conditioned " by ant protection. The mealybugs reach a high level of density in association with the ants, and are maintained at that level by the biotic and abiotic factors discussed above. In this respect Table XXV is of considerable interest. If the lowest mean monthly density is subtracted from the highest mean monthly density and the result expressed as a percentage of the overall mean it is apparent that what will be termed the " degree of oscillation " for the *Sphaerocrema* niche is equal to 69 per cent. of the mean mealybug population for that niche, for other attendant ants it is equal to 111 per cent. and for the no-ant niche it is equal to 264 per cent. of the respective mean populations. Clearly the population occupying the *Sphaerocrema* niche is very much more stable than the populations occupying the other two niches. In fact, it would seem possible that the *Sphaerocrema/P. njalensis* association approaches, as far as the mealybugs are concerned, the " constant environment " referred to by Nicholson (1933, p. 177), in which the density of the mealybugs is caused eventually to reach, and remain at, its steady value.

Unfortunately no quantitative data on relative density of ant populations per tree were collected in the field survey so it is not possible to discuss in detail those factors which control ant population density. There seems little doubt, however, that interspecific competition is mainly responsible for the natural control of ants on cacao. There are, as has been demonstrated, a number of groups of ant species living in the cacao canopy which are negatively correlated one with the other. Certain of these species can live together apparently peacefully on the same tree, but it is rare to find two ant species attending the same Coccid species on one tree. Where ants such as *Oecophylla* and *Crematogaster striatula* occur on the same tree, each ant tending its appropriate Coccid species, the density of the Coccid species appears to be lower than if only one ant/Coccid association was present, and indications are that active competition between the members of the two groups of ants may supervene partly as a result of the Coccids migrating into enemy ant territory in search of new feeding sites.

Swollen shoot affects the density of some of the arboreal ants—more particularly through defoliation rendering the trees unsuitable for those species which build leaf nests, though with *Oecophylla* and *Macromischoides* there is an adequacy of alternative vegetation in devastated cacao farms on which they can build their nests.

In general, the large predatory ants show no aversion to litter migration and re-establishment, after destruction of the nest, is more readily effected than with the important species of *Crematogaster* which do not normally approach ground level. The writer has, in fact, noticed on many occasions that when arboreal Crematogasters are forced to the ground they will try to establish themselves on a new host not by migrating by the shortest possible route over the litter, but in a roundabout manner along fallen twigs and branches. In view of the results set out in Tables V and VI of the first paper (Strickland, 1951) it is evident that the important coccidophilic ants have considerable difficulty in re-establishing themselves after nest destruction and that the situation is not improved by continued attacks by other ant species inhabiting the litter. As such, indications are that natural enemies also play a major part in the control of the mealybug tending ants, and not the least of these enemies is certainly *Oecophylla* which is the dominant ant species inhabiting cacao in the Eastern Province of the Gold Coast. What effect controlling *Oecophylla* would have on the density of the Crematogasterine ants and their associated mealybugs is unknown. The strong negative correlation between the two genera coupled with the dominance of the pugnacious *Oecophylla* would seem to indicate that the latter species tends to control the mealybug tending species. If this is the case, then controlling *Oecophylla* would undoubtedly result in an increase in density of the mealybug tending ants and consequently of the mealybugs as well.

Summary.

A method for the routine quantitative estimation of mealybug populations on cacao trees is described and the results obtained from 24 successive monthly samples are tabulated.

The quantitative results obtained are of such variability that only gross differences in population density are shown as statistically significant.

From a series of analyses based on the survey data the following conclusions have been reached with regard to *P. njalensis*, the most important mealybug vector of swollen shoot virus :—

(a) The species is almost invariably attended in the field by Crematogasterine ants, the most important ant species building protective carton tents over the mealybug colonies.

(b) *Crematogaster* density is closely correlated with mealybug density and the ants construct their carton tents in relation to the numbers of mealybugs to be protected. Some of the arboreal Crematogasterine groups investigated are shown to be consistently associated with higher mealybug populations, and to have a more advanced tent building habit, than others.

(c) Tree to tree variation in mealybug density is shown to be largely dependent on the identity of the dominant ant group.

(d) No direct correlation is apparent between mealybug density and incidence of swollen shoot though populations in areas devastated by virus are usually smaller than in areas in which virus is actively spreading.

(e) The factors of importance in the natural control of *P. njalensis* populations have been discussed in some detail. It is clear that the species is maintained by ant protection at a density level considerably above that prevailing amongst closely related pseudococcid species which are not ant attended to the same extent. At this level of "protected steady density" balance is maintained partly by losses during crop harvesting, and partly by swollen shoot rendering the host plants unsuitable as feeding stations. It is concluded, however, that natural enemies must, in the last analysis, be responsible for maintaining balance.

(f) Since the protected density of *P. njalensis* is a direct result of ant associations it follows that factors tending to control the attendant ant species will have a delayed effect on mealybug density. In this respect it is believed that *Oecophylla*, which is strongly negatively correlated with *Crematogaster* spp., is of importance. Any attempts that may in the future be made to control the mealybugs by killing the ants will have to be designed as specific against the Crematogasterini and non-lethal to *Oecophylla* and the other large predatory ants common on cacao.

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STUDIES ON THE RESPONSES OF THE FEMALE *AËDES* MOSQUITO.

PART I.—THE SEARCH FOR ATTRACTANT VAPOURS.*

By A. W. A. Brown, D. S. Sarkaria† and R. P. Thompson.‡

(Plate V.)

Since vast areas of Canada are heavily infested with various species of *Aëdes* mosquitos, it was considered important to characterise the factors which attract the adult females to warm-blooded animals. These may be separated into questions of convective warmth, infra-red radiation, colour in the visible spectrum, and air-borne vapours. The last factor is dealt with in the present paper, and the other factors will be covered in succeeding contributions. The second paper, immediately following the present one, deals with the assessment of the repellency of air-borne vapours of mosquito repellents.

As far as air-borne vapours are concerned, all workers have agreed on the attractancy of moisture. Studies of the effect of carbon dioxide have led to the conclusion that it is not an attractant but an activator ; nevertheless dry-ice has proved an effective field attractant for *Aëdes* in the U.S.A. (Headlee, 1941 ; Huffaker & Back, 1943) and in Canada (unpublished observations of Mr. J. McLintock, Dominion Entomological Laboratory, Lethbridge, Alberta). The vapour from the human arm has proved attractive, as also has liquid sweat ; but no chemical compounds which are known to be constituents of body secretions have been found to show vapour attractancy. The present investigation is therefore concerned with the attractancy of water vapour, carbon dioxide and candidate attractants, and employs the olfactometer technique to ensure that only the air-borne vapour effect is assessed.

Material.

The insect material consisted of a stock of the yellow-fever mosquito, *Aëdes aegypti* (L.), reared continuously in the laboratory. The larvae were reared in enamel pans on a diet of 1 part dried yeast and 2 parts fox-chow§ (compressed meat-vegetable pellets) which was added in small amounts three times during their larval life.

The adults were kept in a large screened cage, measuring 10 ft. long by 4½ ft. wide by 8 ft. high, erected in a greenhouse. It was furnished with potted plants and strings of raisins to provide food for the males and shelter for both sexes. The population of mosquitos within the cage was maintained at a figure between 1,000 and 3,000. The females were fed once weekly upon rabbits or upon the human arm. The temperatures to which the stock was exposed varied from 23°C. to 33°C., and the relative humidities from 30 to 60 per cent.

Apparatus.

Initial work was concerned with the development of a suitable olfactometer. The Wieting-Hoskins (1939) type of olfactometer, originally developed for housefly

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§ Fox Breeders' Cubes, Master Feeds Company, London, Ontario.

studies and modified for mosquitos by Willis (1947), proved unsatisfactory for this investigation, largely because the insects were confined in so small a cage. A new type of olfactometer was therefore devised, though some features of the Wieting-Hoskins type were retained for treatment of the air supply. It was suspended in the centre of the 360-cu. ft. cage of mosquitos.

The olfactometer used in these experiments consisted of two conical Buchner funnels held 16 ins. apart to serve as ports for the emission of parallel air-streams (Pl. V, fig. 1). They were mounted on a board, 24 ins. long by 8 ins. wide, which was suspended horizontally at a height of 4½ ft. from the ground, in such a way that the ports faced upwards. The mouths of the funnels were 3½ ins. in diameter and were covered by fine black veiling net. They were set in the middle of circular holes cut out of the board and measuring 4½ ins. in diameter, and they projected ¾ in. above the upper surface of the board. On the lower surface, a cylindrical can was attached to fill each hole ; the tube supplying air to the Buchner funnels passed through these cans, and to the bottom of the can was also attached an outlet tube through which the air was sucked away. The air which was emitted from the top of the funnel therefore cascaded over the edge, to be drawn down into the can and away through the outlet tube. The funnels, the cans and the board were painted with black enamel.

The air was supplied from a compressed air cylinder fitted with a reducing valve, its flow regulated from determinations made on a rotameter (Pl. V, fig. 2). The air-stream was then split in a Y, the flow of each of the two streams being checked separately by oil-filled venturi flowmeters. The two streams then passed through glass helices held in a water-bath whose temperature was held by thermostat at 37°C. The two streams were then led by long glass tubes into the cage, and these tubes were connected to the stems of the Buchner funnels in the olfactometer. When the air was to be moistened, gas-washing bottles were inserted in the line ; when it was to be dried, calcium chloride U-tubes were inserted, in both cases before the air was slightly warmed in the water-bath.

Method.

The air-stream was delivered from the cylinder at a rate of 10 litres per minute, and split so that each port was supplied at the rate of five litres per minute. The outlet streams were united and sucked out by a rotary pump at ten litres per minute.

When the air-streams were adjusted, two observers standing outside the cage counted, with the aid of hand tallies, the number of mosquitos that approached within 0·5 ins. of the particular port. No attempt was made to distinguish those mosquitos which returned a second time ; they may have been counted more than once.* As soon as one or other of the observers had reached a count of 50 mosquitos (generally within 2 minutes), they stopped and compared counts. Thereupon the supply tubes were interchanged and the process was repeated.

The counting error of the observers was found to be within 8 per cent., the pair of observers if counting at the same port, finding that, when one had reached the count of 50, the other had counted no less than 46. The position error was considerable, the port on the south side (S) attracting many more mosquitos than the north (N). It was greatest when the olfactometer was in full sunlight ; when the olfactometer was shaded by blankets on the roof and upper sides of the cage, the port on the south side attracted between 21 per cent. and 1 per cent. more mosquitos than that on the north side. Therefore the apparatus was shaded, and all experiments were performed on cloudy days. The position error is compensated by taking the counts in pairs of reversed position ; when the blocks of four paired determinations are so arranged (Table I), the experimental error is below 10 per cent. This is shown for four series of control counts in which moist air was passed through both ports simultaneously.

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* On the average, one-third of the côunts represented returns ; and about two-thirds of those counted actually settled on the ports.

TABLE I.

Control counts made by passing moist air through both ports of the olfactometer.

	First Series		*Second Series*		*Third Series*		*Fourth Series*	
	16–24. II. *Unshaded*		6-7. X. *Shaded*		13. X. *Shaded*		14–15. X. *Shaded*	
	Observer 1	Observer 2	Observer 1	Observer 2	Observer 1	Observer 2	Observer 1	Observer 2
Counts of mosquitos at each of the two ports.	100 (S) 78 (N)	58 (N) 100 (S)	50 (N) 47 (S)	48 (S) 50 (N)	47 (N) 50 (S)	50 (S) 29 (N)	50 (S) 50 (N)	40 (N) 37 (S)
	30 (S) 21 (N)	20 (N) 42 (S)	15 (N) 50 (S)	50 (S) 32 (N)	29 (N) 20 (S)	50 (S) 50 (N)	50 (S) 30 (N)	50 (N) 50 (S)
	54 (S) 44 (N)	18 (N) 69 (S)	50 (N) 50 (S)	48 (S) 8 (N)	50 (N) 49 (S)	38 (S) 50 (N)	50 (S) 50 (N)	35 (N) 43 (S)
	100 (S) 79 (N)	30 (N) 105 (S)	50 (N) 50 (S)	40 (S) 47 (N)			50 (S) 28 (N)	50 (N) 50 (S)
Totals . .	506	442	362	323	245	267	358	355
Ratio . .	100	88	100	90	100	109	100	99

The sex of the mosquitos coming to the olfactometer was established by direct observations confirmed by capturing them and examining under a lens. From examinations made at various periods of the cycle of emergence of the fresh pupae added to the population, it was found that the mosquitos counted at the olfactometer were all females.

Results.

Effect of water vapour.

Two series of tests were made to compare the attractant power of moist air with that of dried air. The use of water in a gas-washing bottle gave relative humidities at the port between 80 per cent. and 90 per cent., as measured by a hair hygrometer placed on the port. The use of the calcium chloride U-tube gave relative humidities at the port between 15 per cent. and 20 per cent.

TABLE II.

Comparative attractancy of moist air and dry air to female *Aëdes.*

First Series			*Second Series*		
Room Conditions :	*Moist Air*	*Dry Air*	*Room Conditions :*	*Moist Air*	*Dry Air*
Temp. 25°C., R.H. 36%	50	7	Temp. 26·7°C., R.H. 65%	50	21
	50	12		50	9
Temp. 24·5°C., R.H. 37%	50	23	Temp. 28·9°C., R.H. 55%	50	12
	50	4		50	11
Temp. 26·7°C., R.H. 48%	50	6	Temp. 29·4°C., R.H. 50%	50	4
	50	15		50	8
Temp. 26·7°C., R.H. 50%	27	50	Temp. 30°C., R.H. 58%	50	6
	50	5		50	11
Total insects at each port	377	122	Total insects at each port	400	82
Ratio	100	32	Ratio	100	20

The figures in Table II show clearly that moist air is more attractive than dry air, since it attracts from three to five times as many mosquitos. The insects would alight and probe on the port emitting moist air, while with dry air they seldom alighted and then only momentarily. It is noteworthy that the second series, performed when the relative humidity of the room was 50–65 per cent., showed no less attractancy for the moist port than the first series, which was run at relative humidities of 36–50 per cent. But in a third series performed at 75–85 per cent. R.H., the moist air-stream attracted only 50 per cent. more than the dry air-stream (450 vs. 259) ; in this case, the mosquitos showed some predilection to hover in the turbulence of the dry air-stream. When the port emitting moist air was compared with the one from which there was no emission although the exhaust suction was still applied, it was twice as attractive irrespective of the room humidity, the factor for moist air vs. no emission being 2·07 at 30–40 per cent. R.H. and 1·94 at 80–85 per cent. R.H. When the port emitting moist air was compared with a " dead " port with no emission or suction, it was 2·2 times as attractive whether the humidity was 80 per cent. or 45 per cent. ; when dry air was compared with a dead port, it was only 0·66 times as attractive, identical results being obtained at 80 per cent. and 45 per cent. relative humidities.

Further tests were made to compare a moist object with a dry one, using either black or white billiard-balls. They were mounted on short posts whose ends were screwed into recesses bored in the balls, and the posts were held in the centre of metal discs. The balls were originally tested by placing them at each end of a glass cylinder 10 ft. long by 12 ins. in diameter. Since the results were not good with the insects confined in this tube, the balls were than compared by placing them 16 ins. apart on the olfactometer board in the large cage. When black balls were used, they were wrapped in a single layer of black silk ; and in the case of white balls, thin cheesecloth was employed. One of the balls was moistened with distilled water, and the other was left dry. Counts were made of the number of landings made by the mosquitos on the balls during a period of one minute : the relative humidity obtained during these experiments ranged between 30 and 50 per cent.

TABLE III.

Comparison of a moist object with a dry one, using either black or white billiard-balls, for attractancy to female *Aëdes*.

	White Object		*Moist Object*		*Black Object*	
	Dry	*Moist*	*White*	*Black*	*Dry*	*Moist*
Number of landings per 1-minute periods.	2	23	4	23	5	33
	5	19	3	9	1	19
	1	7	6	22	1	12
	0	11	4	5	2	19
	4	14	17	82	2	10
	2	9	14	58	1	8
	2	4	10	52	4	31
	1	5	10	24	5	23
	3	25	4	31	6	15
	2	16	3	11	4	28
	2	8	5	32	6	23
	0	7	3	26	6	90
	0	5	6	35	20	56
	1	12			5	75
	1	10			4	85
	0	13				
Totals ..	26	188	89	410	72	527
Ratio ..	10	72	10	46	10	73

R.H. of room 30%–40%.

The results in Table III, obtained at R.H.'s between 30 per cent. and 40 per cent. show that a ball with a moist surface is approximately seven times as attractive as one with a dry surface. This relationship still holds whether the ball is black or white, despite the fact that a black ball is approximately five times as attractive as a white one. On the other hand, when the comparison was repeated at a room R.H. of 80 per cent., the dry black ball was 2·5 times as attractive as the moist black ball ; in this case, males were noted for the first time, alighting on the dry ball, but their numbers were not sufficient to invalidate this result for females.

Effect of carbon dioxide.

Two series of tests were made to assess the attractive power of dry air containing 10 per cent. CO_2 by volume ; this mixture was obtained from a compressed-gas cylinder available commercially. It was dried by passing through a calcium chloride U-tube* and was compared with dry air. The results in Table IV show that the addition of 10 per cent. CO_2 to dry air rendered it more attractive than dry air alone, since it drew 2·2 times as many mosquitos to the environs of the port. When this result was submitted to statistical analysis according to the methods outlined in Mather (1947) a Student's t value of 9·9 was obtained for 15 degrees of variance,† indicating that the probability of obtaining a similar result, or one more pronounced, by pure chance was very much less than 1 in 1,000 ; the difference between the two responses is therefore highly significant.

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* In any case this gas source was found to have a negligible moisture content.

† From the equation $t_{n-1} = \dfrac{\bar{d}}{\sqrt{\dfrac{\Sigma d^2 - 1/n\ (\Sigma d)^2}{(n-1)n}}}$, where d is the numerical difference between a pair of simultaneous counts at the two ports (taking sign into account), and where n is the number of paired counts made and n–1 represents the degrees of freedom.

TABLE IV.

Comparison of 10% CO_2 and 100% CO_2 vapour with air for attractancy to female *Aëdes*.

	Effect of adding 10% CO_2 to Air				*Comparison of CO_2 Vapour with Air*				*Effect of Concentration of CO_2 in Dry Air*	
	When Air is Dry		*When Air is Moist*		*vs. Dry Air*		*vs. Moist Air*			
	Dry Air	*10% CO_2*	*Moist Air*	*10% CO_2*	*100% CO_2*	*Dry Air*	*100% CO_2*	*Moist Air*	*10% CO_2*	*100% CO_2*
Counts of mosquitos at each of the two ports.	48	50	38	50	50	38	17	50	40	50
	15	50	41	50	50	40	28	50	50	20
	40	50	19	50	50	50	16	50	50	18
	36	50	50	42	38	50	19	50	50	29
	21	50	31	50	50	24	20	50	50	18
	39	50	40	50	40	50	11	50	50	29
	13	50	26	50	28	43	20	50	50	33
	24	50	50	33	34	50	8	50	50	15
	22	50	50	46	46	50	25	50	50	42
	21	50	39	50	30	50	13	50	38	50
	14	50	50	52	50	24	34	50	50	50
	18	52	50	50	48	50	21	50	43	50
	23	50	44	50	50	29				
	17	50	50	36	31	28				
	13	50	50	52						
	26	50	45	50						
Totals ..	390	802	673	761	594	576	232	600	571	404
Ratio ..	100	206	100	113	106	100	39	100	100	71

Two series of tests were made to assess the effect of adding 10 per cent. CO_2 to moist air. The figures in Table IV show that the enhancing effect on attractancy was so slight as to be statistically insignificant, the probability of the t value of this being 0·6, or 600 in 1 thousand.

Pure CO_2 vapour from a cylinder was then compared for attractancy with dry air and with moist air. It may be seen in Table IV that the stream of CO_2 vapour happened to be approximately as attractive as the stream of dry air carrying 15–20 per cent. R.H. On the other hand, it proved to be only one-third as attractive as the moist air carrying 80–90 per cent. R.H. ; this result was very highly significant. And it was 30 per cent. less attractive than a dry air-stream carrying 10 per cent. CO_2 ; this result, with a statistical probability of 0·01, or 10 in 1 thousand, was quite significant.

When pure CO_2 is emitted, it is observed that the greater number of the mosquitos stay at distances over 0·5 inches from the port, and are therefore not counted ; they are most commonly found swarming around the black canister underneath the board of the olfactometer. These tests moreover do not fully assess the powerful initial attractancy of CO_2 vapour, nor its observed ability to activate a sluggish population of mosquitos. The question of fall-off in attractancy with time, and of optimum CO_2 tension for attractancy, will be covered in a later communication.

Effect of candidate attractants.

A preliminary search was made for possible attractants for *Aëdes aegypti*, other than CO_2 and H_2O vapour. The materials chosen were either those which might be expected to occur in sweat or urine, such as organic acids and nitrogenous bases, or else those products of fermentation—alcohols, aldehydes and esters, which are known to be attractants for the house-fly. They were dissolved in water in concentrations dictated by their volatility, and the solutions were placed in the bead bubbler. In order to allow for the effect where an attractant may be disguised as a repellent at high concentrations, the substances were tested at two levels, one 100 times the other. The results (Table V) show that so far from proving attractants, all except one of the materials showed repellency ratings at the concentrations

TABLE V.

Materials tested in the olfactometer for possible attractancy.

Concentration in aqueous solution in the bubbler		Number of mosquitos at experimental port for every 100 insects at control port
Acetic acid	10%	40
Acetic acid	0·1%	53
Lactic acid	80%	64
Lactic acid	0·8%	48
Ammonia	2%	48
Ammonia	0·2%	74
Trimethylamine	1%	60
Trimethylamine	0·01%	54
Sweat	6 cc. /armpit	82
Sweat	60 cc. /armpit	141
Ethyl alcohol	10%	82*
Ethyl alcohol	0·1%	74
Benzaldehyde	1%	83
Benzaldehyde	0·01%	92
Ethyl butyrate	1%	99
Ethyl butyrate	0·01%	89
Molasses	50%	72
Molasses	0·5%	88
Beer	100%	58
Beer	1%	100*

* These materials on occasion proved to be significantly attractive.

employed. Statistical analysis showed that ratings of 40 per cent. or more represented highly significant repellency, while the ratings of 28 per cent. or less were of questionable significance. However, ethyl alcohol, ethyl butyrate and beer could give on occasion individual counts that appeared to indicate definite attractancy at the time.

The single material that proved attractant was human sweat, taken from the armpit in filter paper, eluted into a stock solution taken from 11 subjects (both armpits) and diluted so that the product of 1 armpit was contained in 60 cc. of water. This was sufficiently attractive to give 141 mosquitos at the experimental port for every 100 at the control. When 10 times more concentrated it had a repellency rating of 18 per cent. ; when 10 times more dilute it showed an attractancy rating of only 7 per cent. Statistical analysis showed that the attractancy rating of 41 per cent. had a probability of 0·01, and was therefore quite significant, while the repellency rating of 18 per cent. had a probability of 0·04 and thus was of questionable significance.

Discussion of Results.

The decisively attractant effect of water vapour for female adult *Aëdes* observed in these experiments confirms results obtained by other workers. Both Christophers (1947) and Parker (1948) have noted this effect with *A. aegypti* in a glass tube, and Crumb (1922) with *Culex pipiens* L. in a cage. The first-named author observed that the males, on the other hand, were repelled rather than attracted by moisture. It is pertinent that in our experiments we found no males attracted to the moist ports. DeLong and co-workers (Abstr. curr. Inf. Ins. Contr., Nat. Res. Coun., Wash. (N.S.), nos. 3–11, 1946) observed that, with *A. aegypti* in cages, an air-stream was attractive provided its relative humidity was in excess of the surrounding atmosphere, and that the human arm was attractive provided the relative humidity of the surrounding air was 95 per cent. or less, being unattractive at conditions of 100 per cent. saturation. As far as our experiments were concerned, an increase in relative humidity of the room from 36 per cent. up to 65 per cent. involved no reduction in the attractancy of the moist air-stream, but if it was raised to 80 per cent. the attractancy of the moist air-stream was considerably reduced. DeLong and others found that a bowl of water capped by a permeable animal membrane (Baudruche) was attractive ; these experiments show that a simple moist surface is attractive. The figures obtained with both olfactometer and billiard-balls demonstrated the overriding and decisive nature of water vapour as a factor attracting female *Aëdes* mosquitos.

The results here reported for the attractancy of CO_2 in the olfactometer are not inconsistent with results hitherto obtained by other workers. Crumb (1922) observed that the presence of CO_2 in an air-stream did not increase its attractancy to *Culex pipiens*. Willis (1947), using *Aëdes* and *Anopheles* confined in a 0·2-cu. ft. cage in an olfactometer of the Wieting-Hoskins type, found that neither 1 per cent., 10 per cent. nor 50 per cent. CO_2 in moist air was attractive on the basis of instantaneous counts of mosquitos present at the ports. However, DeLong (unpublished report, project 272, Ohio St. Univ. Foundn, Off. Quartermaster-General, Washington, 1949), using larger cages, reported that CO_2 stimulated flight and mating but did not evince an attractive response from *Aëdes aegypti*. Both DeLong and Willis confirm Rudolfs' (1922) original finding that CO_2 is essentially an activator. In the experiments reported above, the addition of 10 per cent. CO_2 to moist air did not increase its attractancy, thus confirming Willis' observations ; but the addition of CO_2 to dry air definitely increased the numbers of *Aëdes* that approached within 0·5 inches of the point of emission. On the other hand, 100 per cent. CO_2 vapour was no more attractive than dry air, and was considerably less attractive than moist air since the mosquitos were kept at a distance from the port. It is felt that the larger the space in which the mosquitos are held, the more CO_2 has the effect of an attractant, so that in the open air of field conditions it is effective enough to act as bait in traps for *Aëdes*.

The present experiments have failed to find any single pure substance which is consistently attractive to adult *Aëdes*. All the compounds showed a repellency rating, which in most cases was statistically significant, and in some cases was as high as the weaker candidate repellents. In this finding there is general agreement with previous workers. Both Crumb (1922) and Rudolfs (1922) found the constituents of human body secretions and excretions to evoke only faint and erratic responses by *Culex* and *Aëdes*. Reuter (1936) found that the various acids to be found in sweat were unattractive to *Anopheles*. DeLong and others reported that the anointing of the moisture-permeable Baudruche membrane with sweat substances such as lactic acid, methyl caprate, sodium acrylate, tributyrin, human fat, etc. did not materially affect the natural attractancy of the warm moist object to *Aëdes*.

The finding that sweat in appropriate concentrations is definitely attractive in an air-stream is in agreement with both Willis (1947) and DeLong, who found that the odour of the human arm, when added to moist air in the olfactometer, was definitely attractive to adult *Aëdes* and *Anopheles*. Moreover, Parker (1948) discovered that human sweat absorbed on a filter paper was significantly more attractive to *Aëdes aegypti* than filter paper moistened with water alone, even when both were at room temperature. On the other hand, Howlett (1910) had found that liquid sweat at room temperature was not attractive to *Aëdes* (*Stegomyia*) or *Culex*: and Reuter found that it was unattractive to *Anopheles*. Other observations on this subject, made by various authors and discussed by Willis or by Parker, are so contradictory that it is evident that this subject is a complex one. Human breath was found by Kennedy (1940) to be attractive to *Aëdes* because it activated them to fly upwind to the source; this author could not visualize any direct means whereby an attractant could orientate free-flying mosquitos.

The present authors are not fully satisfied that concentrations cannot be found at which even the substances here tested could not under some conditions become attractive. Ethyl alcohol occasionally, and ethyl butyrate or beer frequently, appeared to excite a pulse of attraction in the population of mosquitos. DeLong reported that lactic and propionic acids could prove slightly attractive in the olfactometer.

Summary.

The question of the attractancy of water-vapour, carbon dioxide and candidate attractant compounds was investigated by the use of an olfactometer emitting freely into a 360-cu. ft. cage filled with female adult *Aëdes aegypti*. Moist air (85 per cent. R.H.) was found to attract 3–5 times as many mosquitos as dry air (15 per cent. R.H.). A moistened billiard-ball was found to attract about ~~five~~ times as many mosquitos as a dry one, the result being similar whether black or white balls were used. The attractancy was reduced and even reversed at very high room humidities.

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The addition of 10 per cent. CO_2 to a dry air stream approximately doubled its ability to bring mosquitos within 0·5 inches of the olfactometer port. Its addition to a moist air-stream did not significantly increase its attractancy. Pure CO_2 vapour proved to be no more attractive than dry air, and several times less attractive than moist air.

A number of chemical compounds found in body exudations, and some housefly attractants, were tested in the olfactometer for their effect on *Aëdes* adults. None were consistently attractive, and many were significantly repellent. However, sweat itself proved significantly attractive at a low vapour concentration, while significantly repellent at a high concentration.

Acknowledgements.

The authors wish to record their appreciation of the substantial contribution made to this study by Mr. G. T. Crosson, senior technician of the Department of Zoology, University of Western Ontario.

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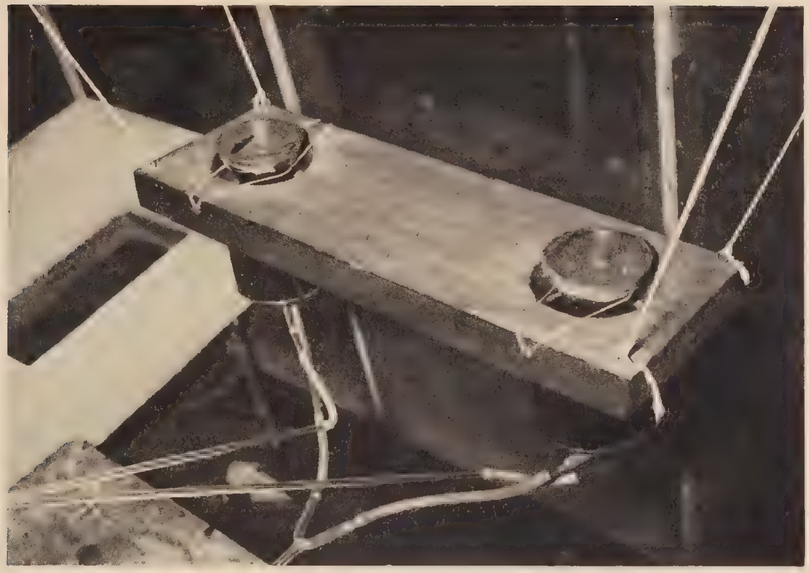

FIG. 1. Olfactometer for testing attractancy of two parallel air-streams to *Aëdes* adults in a 360 cu. ft. cage. Showing lead-in of air-streams through straight glass tubes, Buchner funnels capped with veiling to serve as ports, black canisters to take the exhaust air, which leads to a Y and to the pump.

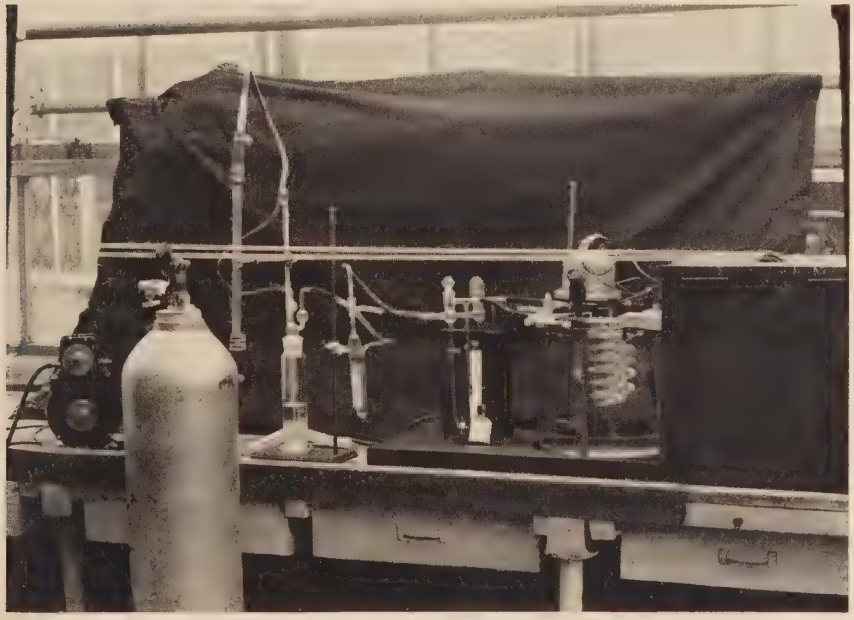

FIG. 2. Air-stream supply to olfactometer and air exhaust therefrom. Left to right: exhaust pump, compressed air cylinder, rotameter, gas-washing bottle, glass Y and bead bubbler, venturi flowmeters, helices in water bath; the long glass tube lead to the olfactometer in the cage.

STUDIES ON THE RESPONSES OF THE FEMALE *AËDES* MOSQUITO.

PART II.—THE ACTION OF LIQUID REPELLENT COMPOUNDS.*

By D. S. Sarkaria† and A. W. A. Brown.‡

Liquid repellents may be divided into two classes on the basis of their mode of affording protection : those like citronellal which are sufficiently volatile to keep the mosquito at a distance, and thus may be designated as vapour repellents (McCulloch & Waterhouse, 1947) ; and those like indalone which are so slightly volatile that they allow the insects to approach and touch the treated skin, and are considered to be mainly contact repellents (Christophers, 1947). Compounds of the latter class therefore are deficient in that they fail to remove the psychological hazard of swarming attack, and allow biting to occur on skin areas left uncovered by the application. Compounds of the former group are deficient in that they are rapidly lost by evaporation from the skin.

The methods hitherto employed to assess the efficacy of repellents are based upon the length of the period over which the inuncted arm remains unbitten. This has the effect of placing emphasis on compounds of the second class, since the most important factor is the lack of volatility, or lowness of the vapour pressure of the compound. However, the degree of persistence may be profoundly modified by the absorption of the material into the skin. Indeed, it has been found impossible to establish a correlation between the volatility of a compound, its persistence on the skin, and the length of the period of repellency (D. M. DeLong, unpublished report, project 272, Ohio St. Univ. Foundn, Off. Quartermaster-General, Washington, 1949).

The experiments here reported represent an attempt to assess the repellent power of the vapours of the various compounds by using olfactometer technique. The approximate vapour pressures of the materials have also been determined. In this way two of the factors, which operated simultaneously in the conventional methods of repellent testing, have been separated for independent study. In addition, in case it might have a bearing on the physiological action of these compounds, their narcotic power has been measured in terms of the speed with which knockdown is induced when mosquitos are exposed to their vapours.

Materials.

The liquid repellents were compounds submitted by synthetic organic laboratories to the Orlando (Fla.) laboratory of the U. S. Bureau of Entomology and Plant Quarantine, whose serial number they bear. A few were tested as Eastman Kodak chemicals or even as commercial retail preparations.

The biological material was a population of adult *Aëdes aegypti* (L.) which had not been fed during the previous 36 hours. Except immediately after the addition of fresh emergents to the cage, the proportion of the more short-lived males was low. It was found that the mosquitos which actually came to the olfactometer were all females.

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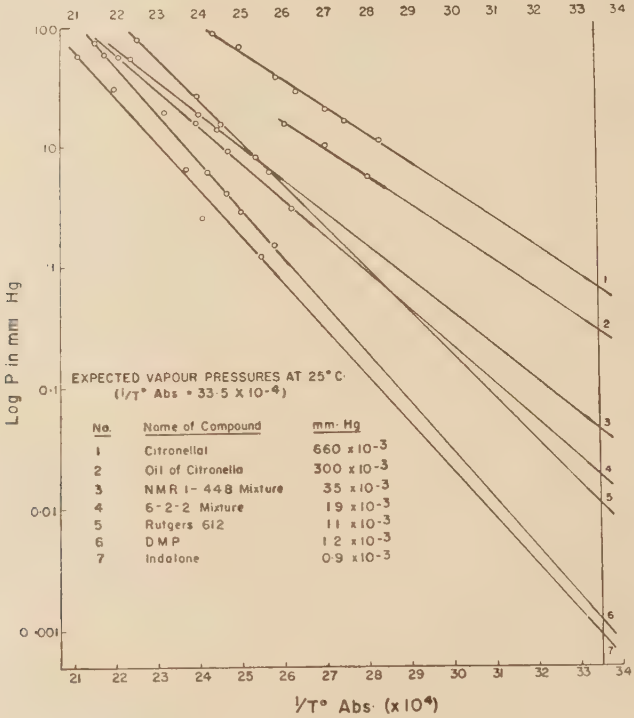
Methods.

The olfactometer employed in the 360-cu. ft. cage has already been described in the preceding paper (p. 106). Both air-streams were humidified by bubbling through water in a gas-washing bottle before being split by a Y-tube and each adjusted to a speed of 5 litres per minute ; they were warmed by passage through helices in a water-bath held at 37°C. The repellent was added to one of the streams by introducing 10 cc. of the liquid into a miniature bead-bubbler placed in the line just before the helix. The relative humidity of the air-streams at the ports was found to be 80–90 per cent. and the temperature to be less than 1°C. above the room temperature. Conditions in the greenhouse room were as follows : temperature 26–29°C., with a maximum range of 24–33°C. ; relative humidity 40–50 per cent., with a maximum of 36–60 per cent.



EXPECTED VAPOUR PRESSURES AT 25° C·
(¹/T° Abs • 33·5 X 10⁻⁴)

No.	Name of Compound	mm· Hg
1	Citronellal	660×10^{-3}
2	Oil of Citronella	300×10^{-3}
3	NMR 1- 448 Mixture	35×10^{-3}
4	6-2-2 Mixture	19×10^{-3}
5	Rutgers 612	11×10^{-3}
6	DMP	$1\ 2 \times 10^{-3}$
7	Indalone	$0{\cdot}9 \times 10^{-3}$

Fig. 1.—Relationship of pressure with the boiling point of certain important mosquito repellents ; and the consequent determination of their vapour pressures at 25° C.

Counts were taken visually of the number of insects which approached within a distance of 0·5 inches of each port. When approximately 50 mosquitos had been counted on the control port carrying the air-stream to which a repellent had not been added, the results were recorded ; then the ports with their connections were interchanged, and the count repeated. At least 4 sets of such double counts were made for each repellent and more for certain of the more important compounds. This involved using the same mosquito population repeatedly ; however, no evidence was obtained that the insects become conditioned to the repellent. Some materials, especially citronellal and citronella oil, required prolonged periods of airing of the apparatus and resting of the mosquitos before the assessment could be completed.

The vapour pressures of the compounds were determined by the Ramsay-Young dynamic method (Daniels & others, 1949), in which the boiling-points were determined at reduced pressures at several stages between 1 mm. and 100 mm. Hg. As many points on the pressure-boiling point curve were determined as the limited amount of material allowed, but the figures obtained may only be regarded as approximate. By plotting the boiling point as the reciprocal of the absolute temperature against the logarithm of the pressure (or the pressure on a logarithmic scale), a straight line is obtained which allows extra-polation to find the expected vapour pressure of the compound at 25°C. The pressure-boiling point relationships of the most important repellents are shown in fig. 1.

The narcotic power of the repellents was determined by the use of 20-litre glass bottles. Their bases were warmed and 0·05 cc. (approx. 50 mg.) of the liquid repellent was added by pipette. After the repellent had been allowed to volatilize for one hour in the closed space, a cylindrical cage containing 20 mosquitos was suspended in the middle of the bottle. By periodic observation it was possible to establish the time at which 50 per cent. of the insects were knocked down (KT_{50}) and similarly the KT_{80} and KT_{100} figures. The KT_{80} was chosen as the basis of comparison, and the figure was established in view of the three measurements taken for each compound ; usually it was approximately twice the KT_{50} figure and half the KT_{100} figure. Control experiments, in which the mosquitos were treated similarly but without repellent, showed that 50 per cent. mortality did not usually occur before 24 hours had elapsed ; since, however, the lowest control figure obtained was KT_{80} of 14 hours, repellents showing a KT_{80} above 12 hours are classed as negative with respect to knockdown.

Results.

The olfactometer assessments showed that all of the 42 candidate repellents tested showed vapour repellency, and in 39 of them this effect was quite pronounced. The results obtained with the more powerful repellents in general use are shown in Table I. It may be seen that the vapour repellency of citronellal, for example, was such that only 19 mosquitos came to the treated air-stream for every 100 that approached the untreated port ; the repellency rating was therefore taken as 81 per cent. for those particular determinations. Repellency ratings were obtained for the other compounds in a similar manner. Owing to the day-to-day variation in response, significance may be attached only to a grouping of the compounds into five classes according to their repellency (Table II).

The results for the four least repellent compounds were submitted to statistical analysis for significance. The repellency rating of 34 per cent. for diethylundecylenamide (0–7146–a) was found to involve a Student's t value of 0·001*, indicating that the probability of this difference being due to chance was 1 in 1,000. Therefore it is valid to conclude that compounds in class four and the preceding classes are very significantly repellent. The Student's t values for the three compounds in class five were as follows : 0·045, 0·08 and 0·35. Therefore it is concluded that the repellency rating of 15 per cent. for isoamylacetanilide is not significant, while the repellency of the other two compounds is of questionable significance.

All of the repellents in practical use fall into the first or second class, with vapour repellency ratings in excess of 60 per cent. The high degree of vapour repellency of citronellal is emphasized in these results ; this is considered by Christophers to be almost certainly the chief active agent in citronella oil, although methylheptenone (b.p. 174°C.) has been quoted by Wood and Osol (1943) as the main repellent principle.

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* From the equation $t_{n-1} = \dfrac{\bar{d}}{\sqrt{\dfrac{\Sigma d^2 - 1/n\ (\Sigma d)^2}{(n-1)n}}}$, where d is the numerical difference between a pair of simultaneous counts at the two ports (taking sign into account), and where n is the number of paired counts and n–1 represents the degree of freedom.

TABLE I.

Repellency assessments of the more important repellents for female *Aëdes aegypti*.

Repellent	*Citronellal*		6–2–2*		*Citronella*¶		*Indalone*†		*Rutgers* 612‡		*NMRI*-448§		*DMP*‖	
	Air	*Chem.*	*Air*	*Chem.*	*Air*	*Chem.*	*Air*	*Chem.*	*Air*	*Chem.*	*Air*	*Chem.*	*Air*	*Chem.*
Counts of mosquitos at control port (Air) and at port emitting the repellent (Chem.).	52	19	52	13	50	22	51	24	52	16	50	23	57	22
	50	4	54	16	50	5	52	36	50	8	53	11	53	49
	51	11	50	10	50	14	52	32	50	10	53	26	52	17
	31	6	50	11	50	19	51	25	50	23	50	8	47	40
	50	13	50	10	50	11	50	18	70	23	50	22	50	27
	58	4	51	9	50	18	50	25	50	21	50	18	52	24
	50	16	50	16	50	8	50	6	51	14	50	22	50	5
	53	3	50	14	50	30	50	18	50	38	50	20	50	18
			50	10	50	7	50	11					50	5
			50	8	50	20	50	12					50	18
			50	16	50	12	50	6					50	9
			50	15	50	17	50	16					50	16
							46	23					50	11
							50	5					50	6
Totals ..	395	76	607	154	600	183	702	245	423	153	406	150	711	267
Ratio ..	100	19	100	25	100	30	100	35	100	36	100	37	100	38
Repellency Rating ..	81%		75%		70%		65%		64%		63%		62%	

* A mixture of 6 parts DMP, 2 parts Rutgers 612, and 2 parts indalone.

¶ Ceylon oil of citronella.

† 1, 2-pyran-6-carboxylic acid, 3, 4-dihydro-2, 2-dimethyl-4-oxo-butyl ester.

‡ 2-Ethyl-hexanediol-1,3.

§ A mixture of 7 parts of 2-phenylcyclohexanol with 3 parts of 2-cyclohexylcyclohexanol,

‖ Dimethyl phthalate.

Repellent power, narcotic speed, and vapour pressure of liquid repellents for female *Aëdes aegypti*.

No.	Name of Compound	Observed Repellency Rating	Vapour Pressure mm. Hg. × 10^{-3}	Knockdown KT_{80} in hours
Repellency Rating 71–85%				
E.K. Pract.	Citronellal	81	660	0·5
0-4202-a	n-Hexyl mandelate	80	25	7
0-4262-b	Isobornyl 4-morpholinoacetate	78	1·5	Neg.
6-2-2	DMP - Rutgers 612 - Indalone	75	19	6
0-301-a	n-Hexyl diethyleneglycol monoether	73	5·5	1·0
Repellency Rating 61–70%				
Commercial	Oil of citronella (Ceylon)	70	300	0·5
0-3572-b	Di-isopropyl tartrate	67	1·2	10
0-9-h	Indalone	65	0·9	12
Rutgers 612	2-Ethyl-hexanediol-1, 3	64	11·0	Neg.
0-1023-f	2-Cyclohexylcyclohexanol	64	0·5	Neg.
0-3916-h	Dimethyl carbate (heptane)	64	5·4	1·5
NMRI-448	Phenylcyclohexanol-cyclohexylcyclohexanol	63	35	2·5
0-5567-c	Diethyl hexahydrophthalate	63	4·2	0·9
0-6154-d	Pentamethylene dipropionate	63	1·5	1·0
0-5533-b	4-Anisyl-5-methyl-m-dioxane	63	1·7	1·0
DMP	Dimethyl phthalate	62	1·2	8
0-4561-a	2-Ethylhexyl glyceryl ether	62	0·1	6
0-5563-d	β-Tetralol	61	25	1·0
Repellency Rating 41–60%				
0-6216-c	Ethyl-β-phenyl-β-hydroxy-propionate	60	15	Neg.
0-2133-h	2-Phenylcyclohexanol	60	2·6	0·2
0-4203-a	Isobutyl mandelate	59	33	0·1
0-3916-k	Dimethyl carbate (heptene)	58	7·0	0·3
0-4145-c	1, 2, 3, 6-Tetrahydrophthalimide	54	20	8
0-614-c	Indimide (N-cyclohexylbutoxy-acetamide)	54	3·3	Neg.
0-6154-b	Pentamethylene propionate	49	1·5	6
0-925-b	Indimide A (N-amylbutoxy-acetamide)	48	33	0·1
E.K. Chem.	n-Caprylic acid	48	5·5	2·5
0-7126-a	Undecylenic acid	48	0·8	10
0-5542-b	2, 2-Thiodiethanol diacetate	48	1·1	3
0-5518-c	p-n-Propoxybenzaldehyde	47	0·9	0·2
0-300-a	2-Ethylhexyl diethyleneglycol monoether	46	5·5	Neg.
0-7090-c	5-Ethyl-5-nitro-2-propyl-m-dioxane	43	3·2	2·5
Repellency Rating 30–40%				
0-6378-a	1, 3-Propanediol benzoate	40	0·1	Neg.
0-6484-a	Methyl N, N-diisopropyladipamate	39	0·01	6
0-6252-b	Ethyl N, N-dipropylsuccinamate	38	1·0	4
0-7090-a	Butanal 2-nitro-2-ethyl-1, 3-propanediol acetal	35	5·0	5
0-7416-a	N N-diethylundecylenamide	34	0·9	5
Repellency Rating less than 25%				
0-6257-a	1, 3-Cyclohexanediol butyrate	24	1·8	2·2
0-6358-a	Methyl N, N-diisopropyladipamate	18	2·0	7
0-5528-b	N-isoamylacetanilide	15	2·3	0·2

The vapour pressures of the candidate repellents at 25°C. were found to range from $0{\cdot}01 \times 10^{-3}$ mm. Hg. up to 660×10^{-3} mm. Hg., with the most frequent figures lying between 1 and 10×10^{-3} mm. Hg. The average situation may be exemplified by DMP, whose vapour pressure is expected to be $1{\cdot}2 \times 10^{-3}$ mm. Hg. at 25°C., which was found to have a boiling point of 114°C. at 1·5 mm., 193°C. at 74 mm., and 282°C. at 760 mm. Hg. (see fig. 1). The figures obtained for citronellal confirm the correctness of the boiling point figure of 208°C. at 760 mm. quoted in the Handbook of Chemistry and Physics* and suggest that the figure of 80·7°C. cited by Christophers (1947, p. 198) must be a misprint.

Although there is a tendency for the more volatile compounds to fall in the higher class of repellency rating, there is no regular relationship to suggest that vapour repellency is simply a measure of the volatility of the compound and thus of the parts per million taken up by the air-stream. It is true that all of the compounds whose vapour pressures exceed 10×10^{-3} mm. Hg. are to be found in the first three classes; nevertheless compounds with pressures as low as $1{\cdot}5 \times 10^{-3}$ mm. occur in the first class, and the second class contains compounds with pressures ranging down to $0{\cdot}1 \times 10^{-3}$ mm. Hg. Thus the repellency as measured does indicate the particular potency of the compound, and is not just an artifact based on the degree of volatilization in the bubbler.

The fumigation experiments show that the vapours of all except seven of the repellent compounds have narcotic power. In most cases a period of hyperactivity preceded knockdown, but it was impossible to state whether this was due to the irritancy of the vapour or to the initial stimulatory effect characteristic of a narcotic. Some compounds caused 80 per cent. knockdown within 30 minutes (*e.g.* citronellal); or even 10 minutes (*e.g.* indimide A). Although some of these faster narcotics are highly volatile, others are not (*e.g.* propoxybenzaldehyde), and although some are good vapour repellents, others are not (*e.g.* isoamylacetanilide). The repellents whose vapours were not narcotic were characterized by slight volatility, with the notable exception of Rutgers 612.

Discussion of Results.

These results indicate that vapour repellency is a characteristic of mosquito repellents, and that the well-known effective repellents fall into the classes with the highest vapour repellency. Nevertheless, this does not imply that the less volatile compounds, such as indalone, may not function in practice chiefly as contact repellents, as Christophers has stated. It is noteworthy that he found indalone and DMP to have sufficient vapour repellency to shift the *Aëdes* population in a tube so that there were 40–50 per cent. more at the distal end than at the proximal end where the repellent was presented. In his experiments Rutgers 612 gave 2·5 times as many mosquitos at the distal end as the proximal, whereas citronellal raised this figure to 45 times. DeLong and others (Abstr. curr. Inf. Ins. Contr., Nat. Res. Coun., Wash. (N.S.), nos. 3–11, 1946) also found DMP and Rutgers 612 to be vapour repellents, and classed indalone and isopropylsuccinnamate as tactile repellents.

The rating of these compounds on the basis of the repellent power of their vapour, made in this investigation, is in general agreement with the rating already obtained from the conventional tests of their longevity on the surface of the arm or leg, or on clothing. Citronellal and citronella oil are outstanding, due mainly to their high volatility; that they also have a high repellent power is testified by their excellent performance in northern latitudes where lower skin temperatures allow them to persist for a longer period (Pijoan, 1947). Dimethyl phthalate, indalone and cyclohexylcyclohexanol show a high vapour repellency despite a low volatility and thus a low yield of vapour to the air; had they been more volatile their vapour repellency would undoubtedly have been very high indeed. The combination of a high vapour repellency rating with a low vapour pressure makes for a powerful combination. Such an instance is given by isobornyl morpholinoacetate, which has proved to be outstanding in durability as an anti-mosquito clothing impregnant.* Another vapour repellent of the first class is the moderately volatile n-hexyl mandelate, which proved to be the most effective clothing impregnant against *Aëdes* in Alaska (Travis, 1949). Both n-hexyl mandelate and n-hexyl diethylene glycol monoether gave exceptionally long protection when impregnated in stockings and uniforms and tested against *Aëdes* at Churchill on Hudson Bay (H. F. Cross & others, Interim Rep. Surg. Gen. Off., Wash., no. 0–138, 1950). 6–2–2 mixture is generally recognised to be an excellent skin repellent, and the results show that this property is due to a high vapour repellency combined with a moderate volatility. These olfactometer results confirm that the formula NMRI–448 and its constituents (Pijoan, 1947) are less effective than the formula 6–2–2 and its constituents. However, the NMRI constituent 2-phenylcyclohexanol has been found to give longer skin protection than Rutgers 612 (Travis & others, 1949). It is also stable to rinsing when used as a clothing impregnant, but is not as powerful a repellent as DMP or Rutgers 612 for this purpose (Linduska & Morton, 1947).

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* Chemical Rubber Publishing Company, Cleveland, Ohio, U.S.A.

The finding that DMP vapour has a comparatively slow knockdown effect while that of phenylcyclohexanol is faster, is in agreement with results reported by DeLong and others. These investigators also established that contact application of DMP, phenylcyclohexanol, Rutgers 612 or indalone caused knockdown of mosquitos in 30 minutes. They noted the vapour knockdown was reversible if the insects were removed to fresh air soon after complete narcosis. In the present experiments, when the mosquitos were removed from the bottles as soon as 100 per cent. knockdown had occurred, the recovery rate was found to range between 15 and 100 per cent. in the cases where the KT_{100} was four hours or less; with those vapours which required eight hours or more for complete knockdown, there was no recovery.

Summary.

A number of liquid mosquito repellents were assessed for vapour repellency power in an olfactometer mounted in a very large cage filled with females of *Aëdes aegypti*. They were also tested for their knockdown power in fumigation bottles. Their vapour pressures were determined by the Ramsay-Young method.

All the liquids showed vapour repellency, and in 39 out of the 42 tested this effect was highly significant. The highest vapour repellency ratings were shown by compounds already known to be the most effective repellents.

Although the more volatile compounds such as citronellal tend to show the highest repellency ratings, nevertheless compounds of low vapour pressure such as indalone, DMP and isobornyl morpholinoacetate may also show high vapour repellency. It is concluded that vapour repellency, although in the first instance dependent upon volatility, can vary independently of vapour pressure, so that compounds may be found which afford not only a long protection period due to their non-volatility, but also a high vapour repellency due to the potency of the comparatively few molecules that are volatilised.

The vapours of most of the repellents were found to induce knockdown of mosquitos, but there was no correlation between the speed of this process and the vapour repellency of the compounds.

Acknowledgements.

The authors wish to thank Mr. H. F. Cross and Prof. A. A. Kingscote for their generosity in making many of the compounds available, and Dr. E. F. Knipling and Dr. W. V. King, of the U.S. Bureau of Entomology and Plant Quarantine, for their courtesy in allowing the free international passage of these materials.

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G. Special acknowledgment is made of the outstanding services performed by Mr. T. Crosson, senior technician of the University department, during the progress of this investigation.

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THE RECORDED DISTRIBUTION OF THE TICK *RHIPICEPHALUS SANGUINEUS* (LATREILLE).

By H. S. Leeson.

London School of Hygiene and Tropical Medicine.

Information about the recorded distribution of the tick, *Rhipicephalus sanguineus* (Latr.) was accumulated in the same way as that gathered and mapped by Buxton (1941) for certain fleas. The search of the literature which was begun by Miss Benzian was continued by me to January, 1949. Besides the examination of original papers the following were consulted: *Review of Applied Entomology*, Series B, *Tropical Diseases Bulletin* and *Zoological Record.*

R. sanguineus was first described and figured by Latreille in 1806 as *Ixodes sanguineus* from France. For nearly 100 years the tick was only infrequently mentioned in scientific literature, sometimes by other names, until Neumann's monograph appeared in 1911. During this period it was recorded from East Africa as *Rhipicephalus stigmaticus* Gerst.; later this " characteristically African " tick, as it was called, was said to occur not only in Africa and France but also in Italy, in Asia and in Central and South America but no mention was made of North America until 1908 when Banks referred to the tick as *Rhipicephalus texanus*, from Texas and New Mexico.

From this time on, the tick has been mentioned more and more frequently and reports of its presence have come from so many countries that in 1946 Cooley called it the most widely distributed tick in the world. It has been found on a variety of animals but its favourite and normal host is the domestic dog.

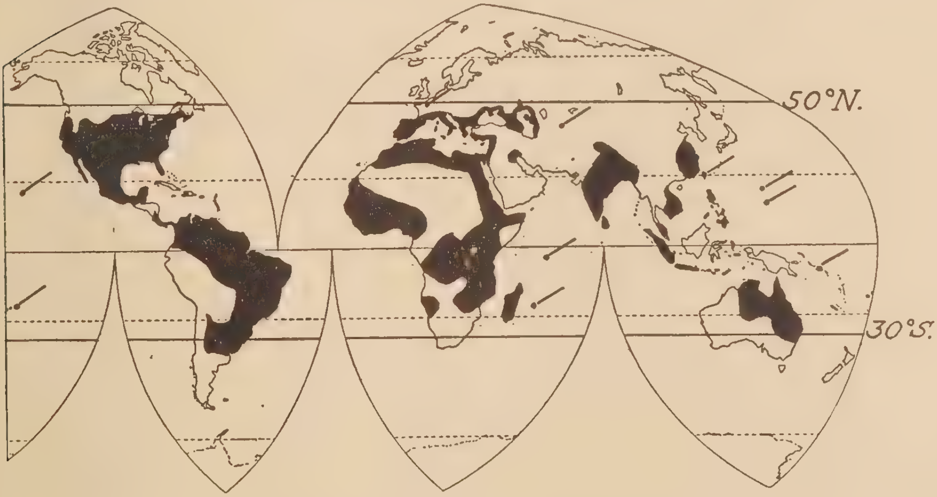

Fig. 1.—Recorded distribution of *Rhipicephalus sanguineus* to January, 1949.

On the map, the black areas cover localities where *Rhipicephalus sanguineus* has been reported to occur. An isolated record is shown by a black dot at one end of an oblique line.

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THE MOVEMENT OF GRAIN WEEVILS THROUGH GRAIN.

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(Plate VI.)

The work described here* was undertaken to obtain information on the probable movements of *Calandra* in grain stored in bulk. Howe (1943) had demonstrated an upward movement of population in a heating bin of grain while D. P. Pielou (unpublished report, 1940) had shown that in narrow vertical glass tubes (4 ft. x 1 in.) weevils moved up if placed at the bottom but did not move down from the top. Nevertheless, under commercial conditions it is the centrally placed population which is most likely to cause heating and it is therefore important to know whether, and under what conditions, weevils will penetrate to the centre of a bulk of grain, and whether, if originally placed there, they will remain in that position.

The majority of the experiments were carried out in metal " towers ". These are sections of iron piping 26 ins. long and 4 ins. in diameter, which can be fitted together. Most of the experiments were carried out with towers of one or two sections, called " short " and " long " respectively. The joint between the sections of piping in long towers was sealed with plasticine.

When set up, both ends of the towers were covered by a double-thickness of muslin, secured by rubber bands.

A special sampling frame (Pl. VI) was built which consists essentially of clamps to hold the tower in position over a sliding block 2 ins. deep. This block rests on a fixed platform and can be moved inwards so that a hole in it (4 ins. diameter) can be brought immediately under the tower. The grain in the tower then falls bodily, filling the hole in the block. Pulling the block out again seals the bottom of the tower and carries the grain sample over a hole in the platform through which the grain falls into a sampling tin.

It is essential to fix the tower so that it just touches the block without making it impossible to remove the muslin from the bottom of the tower. The back edge of the hole in the block is reinforced by metal to prevent jamming.

When a tower is being sampled a few weevils generally cling to the muslin at the top of the tower. These are knocked off and added to the top sample. The maximum number of samples yielded by long and short towers is 26 and 13, respectively.

To determine the accuracy of sampling, the 5th, 10th, 15th and 20th sections, numbering from the bottom, were made up of samples of stained grain. Samples were taken in the ordinary way and then passed through a sample divider so that 2,000–3,000 grains were obtained at random. The percentage of unstained grains in the samples is given in Table I. This shows that the sampling is rather less accurate at the upper levels.

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* This work was part of the programme of the Pest Infestation Laboratory, and is published by permission of the Department of Scientific and Industrial Research.

TABLE I.

Efficiency of sampling towers.

Stained sample No.	% of unstained grains in sample	% of stained grains in:	
		sample above	sample below
5	22·7	6·4	9·0
10	29·4	6·2	18·5
15	35·5	10·8	23·8
20	44·6	7·3	26·6

The pressure changes in these towers are negligible. Pressure increases to a depth of three times the diameter of the tower and then remains constant. Packing probably increases with depth but was not measured in these experiments. Pielou (unpublished report, 1940) measured the packing of grain in a square tower 8 sq. ins. in cross section and estimated a change from 1,300 grains per 100 cc. at 10 cm. depth, to 1,800 at 230 cm., an increase of nearly 40 per cent. Jones (1943) showed that the air space in grain changed from 44 per cent. when the grain was loosely packed to 36 per cent. when the packing was tight, which again is equivalent to an increase of 40 per cent. in the number of grains per 100 cc.

Towers were set up on a bench in the laboratory and carried carefully to a dark constant temperature and humidity room, 25°C. and 70 per cent. R.H., where they were stood on the floor. Although this was always done carefully the effect of this journey on the packing was apparently somewhat variable.

Experiments with Weevils in Towers containing Grain of Uniform Moisture Content.

Preliminary experiments to show the trend of weevil movement in grain were performed in the towers described above, in grain of uniform moisture content, the weevils being placed either on the top, at the bottom or in the middle of the grain column. The experiments were usually allowed to run for a week. Shorter periods produced very irregular results, probably because the insects were still migrating (fig. 1). The longest experiment lasted for four weeks, but at the end of this period larvae which hatched from eggs laid at the beginning of the test would be large enough to be producing appreciable quantities of heat, thus introducing additional complications.

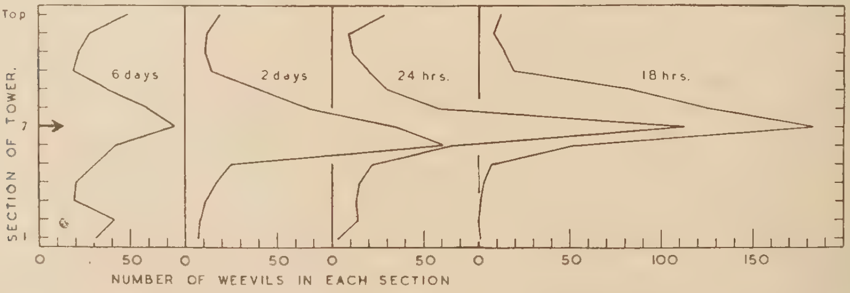

Fig. 1.—Position of weevils in towers broken down 18, 24 and 48 hours, and six days after being set up. Weevils originally in section 7.

The most striking and unexpected result was the tendency for the weevils to move down the tower rather than up.

When weevils were placed at the top of the grain column there was considerable penetration and the majority of the weevils (Table II) moved down out of the top section (2 ins. depth). In some of the experiments in which weevils were placed at the base or middle of the grain column, movement was very slight but in others there was considerable migration (Tables III and IV). A few weevils often managed to move a long way even when the movement of the majority was slight, and usually one weevil, of those originally put in the middle, reached the top sample.

TABLE II.

Movement of weevils placed at the top of a column of grain.

(Moisture content of grain in each experiment between 13 and 14%.)

% left in top section	19	25	27	32	43
Total No. sections	12	24	11	26	26
Furthest section down reached by a weevil	11	24	11	14	19

TABLE III.

Movement of weevils placed at the bottom of a column of grain

(Moisture content of grain in each experiment between 13 and 14·5%.)

% left in bottom section ..	13	15	16	29	53	55	84	86	90	93	95	98	98	99	100
Total No. sections	13	24	13	13	24	24	11	24	25	11	25	25	13	13	13
Furthest section up reached by a weevil ..	13	19	13	13	11	23	6	10	9	3	11	11	13	13	1

TABLE IV.

Movement of weevils placed in the middle of a column of grain.

(Moisture content of grain in each experiment between 12·5 and 16%.)

% in middle 3 sections ..		32	40	46	52	56	62	71	80	83	91	94
% moving up		9	34	38	17	15	19	9	11	9	6	3
% moving down		59	26	16	31	29	19	20	9	8	3	3
% reaching end sixth of tower ..	top	0·6	27·5	26·6	12·7	7·3	3·4	1·2	3·0	0·6	1·0	0
	bottom	10·9	18·1	0	8·1	10·4	0·2	4·1	0	1·5	0	0

In later experiments, weevils were spread evenly through the grain column. In these also the weevils moved to the ends from the middle and generally preferred the bottom to the top.

If the towers containing grain were placed horizontally, weevils moved rapidly from the middle of the grain column to the ends. If weevils were originally placed at one end the amount of movement was reduced but some individuals reached the other end. Movement was considerably more rapid in horizontal than in vertical towers presumably on account of the looser packing.

Effect of Size of Insect on Movement.

In some towers equal numbers of *C. granaria* (L.) and *C. oryzae* (L.) were used. Usually the pigmy strain of *C. oryzae*, which is much smaller than *C. granaria*, was used but on a few occasions the large strain, which is a little bigger than *C. granaria* was chosen. The pigmy *C. oryzae* was markedly more mobile than *C. granaria*, which in turn was slightly more mobile than the large *C. oryzae*. Pigmy *C. oryzae* showed an even greater tendency than the others to move downwards (Table V). This difference persisted at all moisture contents and points to size being an important factor in movement in the grain column.

This fact led to an examination of the effect of size of individuals of a species on the movement of that insect. In one long tower the weevils found in each section were weighed, individually when ten or fewer were found, and collectively for the larger groups. The mean weights of weevils were high in the central and top sections of the tower and low in the bottom sections (fig. 2). Nevertheless, some heavy

TABLE V.

Movement of mixed *Calandra* species put at middle of column of grain.

No. of sections		23	23	23	11	12	12	12	12	23	23	13		24	12	12
% in middle 3 sections ..	*g* *p*	68 40	68 29	67 29	69 56	59 11	79 43	63 18	44 7	13 10	29 13	74 26	*g* *o*	84 94	54 76	55 57
% moving up	*g* *p*	11 3	10 6	21 34	4 6	9 4	5 6	27 14	34 27	15 5	10 3	3 3	*g* *o*	3 6	14 8	2 8
% moving down ..	*g* *p*	21 57	22 65	12 37	27 38	32 85	16 52	10 68	22 66	72 85	61 84	23 71	*g* *o*	13 0·4	32 16	43 35

g = *C. granaria*. *o* = *C. oryzae*. *p* = pigmy race of *C. oryzae*.

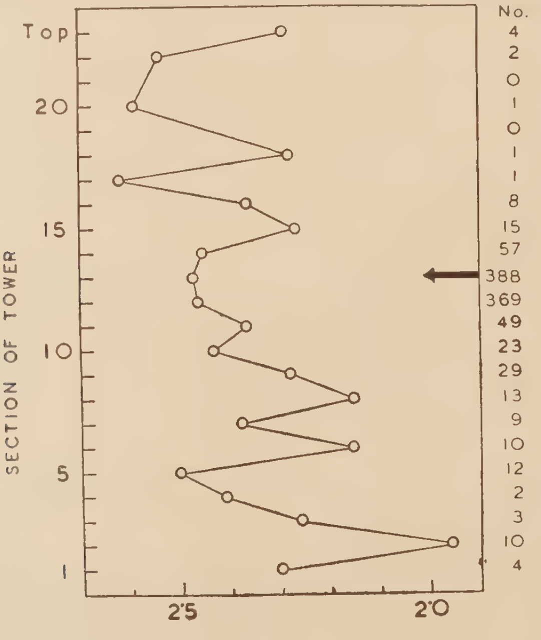

Fig. 2.—Mean weight of weevils plotted against their position in a long tower. The number found in each section is given. Weevils originally in section 13.

individuals had moved downwards and some light ones upwards. The insects used in this experiment were of mixed ages. The weight of insects not having access to free water decreases with age, so weight is not a good measure of linear size unless the insects are of similar age. Consequently some experiments were made using insects less than two weeks old, weighed and sexed at emergence. These were divided into groups, light and heavy, of similar sex ratio, and used in similar short towers. These experiments, however, did not give consistent results.

The Influence of Moisture Content.

Jones (1943) has shown that increased moisture content of grain is accompanied by an increase in the air space in the grain. This suggests that there should be more freedom of movement in damp grain and in fact (fig. 3) there was normally more migration in grain of higher moisture content.

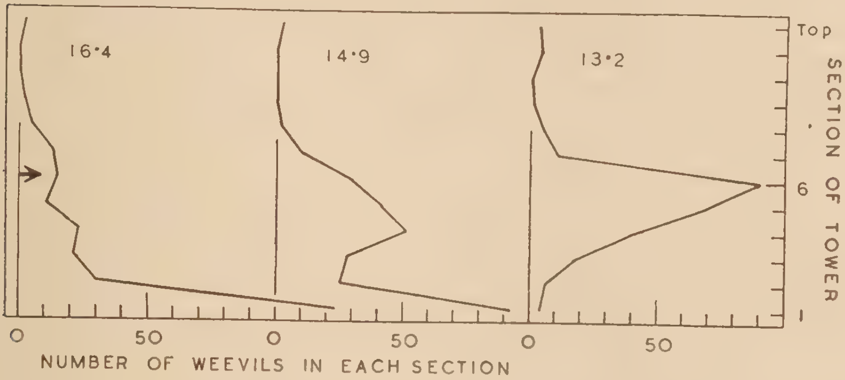

Fig. 3.—Effect of grain moisture content on weevils placed in section 6 of short towers.

The majority of the experiments were performed with an outside humidity of 70 per cent. R.H. and as far as possible most experiments were done with grain of about 14 per cent. moisture content—*i.e.*, in equilibrium with a relative humidity of 70 per cent. When the moisture content of the grain had to be different, as when the effect of moisture content itself was being tested there was necessarily some slight humidity gradient in the tower and some change of grain moisture content at the tower ends. Moisture content as measured at the completion of experiments revealed maximum differences of about 0.5 per cent.

The humidity gradient had no obvious effect on the collection of insects at the ends of towers.

Choice of Moisture Content.

One experiment was carried out in a short horizontal tower with *C. granaria* and pigmy *C. oryzae* placed in the middle between dry (13.4 per cent.) and wet grain (14.9 per cent.). Table VI gives the results of this experiment. Similar experiments were performed in pairs with short vertical towers using *C. granaria* only. Table VII summarises the results of these experiments.

TABLE VI.

Redistribution of weevils when placed between wet and dry grain in a horizontal tower.

	Whole Tower			End Sections	
	Wet half (6 sections)	Dry half (6 sections)	Middle (1 section)	Wet (3 sections)	Dry
C. granaria ..	61 (22·3%)	191 (69·7%)	22 (8·0%)	46 (16·8%)	160 (58·4%)
C. oryzae .. (pigmy)	100 (48·5%)	83 (40·3%)	23 (11·2%)	39 (18·9%)	46 (22·3%)

TABLE VII.

Movement of *C. granaria* placed between wet and dry grain in two vertical towers, the wet grain occupying the upper position in one and the lower position in the other.

Movement after	Distribution within						
	Whole Tower of 13 Sections					Extreme Ends	
	Wet half (6 sections)		Dry half (6 sections)		Middle (1 section)	Wet (3 sections)	Dry
	No.	Position	No.	Position			
4 days ..	15·4% m/c		13·4% m/c				
	181	Upper	168	Lower	147	48 Up.	38 Lr.
	311	Lower	81	Upper	102	71 Lr.	12 Up.
7 days ..	14·5% m/c		13·2% m/c				
	99	Upper	340	Lower	39	38 Up.	259 Lr.
	255	Lower	159	Upper	80	118 Lr.	63 Up.
14 days ..	15·3% m/c		13·9% m/c				
	101	Upper	342	Lower	10	73 Up.	321 Lr.
	288	Lower	99	Upper	22	231 Lr.	74 Up.

TOTALS

	Moisture content factor		Position factor	
	Wet	Dry	Lower	Upper
4 days ..	492 (119)	249 (50)	479 (109)	262 (60)
7 days ..	354 (156)	499 (322)	595 (377)	258 (101)
14 days ..	389 (304)	441 (395)	630 (552)	200 (147)

These experiments provide further evidence of the attraction of the ends, especially of the bottom of the towers. The moisture content results, however, are contradictory. The contradictions probably arise from two causes, the internal physiology of the weevils and the attraction of " ends ". Pielou (unpublished report, 1940), has shown that weevils ordinarily prefer high humidities but that weevils bred in very damp cultures at first prefer low humidity. After about four days this reaction is lost and high humidities are preferred. Inspection of the data concerning the insects used in these towers showed that whenever insects were obtained direct from a culture, a preference for dry grain was shown. When the test insects had been previously used in another tower experiment, wet grain was preferred.

The preference for the end of a tower overrides the effect of moisture content. Consequently in these tower experiments, if the weevils move to dry grain for four days, they remain in the dry grain and move to the " end " even after their humidity reaction had become reversed.

Some more complex experiments were tried using three or four different moisture contents simultaneously. If four different moisture contents were offered, four towers were set up at the same time and each moisture content was placed in each of the four positions in the grain column. The weevils were distributed evenly throughout the towers.

Table VIII gives results of a four-day experiment with six vertical towers, using three moisture content levels; of a four-day experiment with vertical towers, using four different moisture contents; of one 14-day experiment with horizontal towers, and of two with vertical towers, all using four different moisture contents.

The results of these experiments are compatible with the hypothesis that weevils prefer damp grain. There is some suggestion that in short experiments the effect of moisture contents shows best but that in longer experiments the attraction of " ends ", a more progressive and overriding effect, masks it.

In the first of this batch of experiments, the top third of the tower was slightly more populated than the bottom third in the totals. With wet grain in the middle some weevils moved to the middle from the ends, but the movement out of dry grain in the middle was very rapid. On the other hand, at the ends of the towers dry grain was chosen in preference to wet.

Other Experiments.

As the direction of movement in grain was contrary to expectation, to field observation and to the results of Pielou (unpublished report, 1940) it was thought that the sampling apparatus might be at fault. For instance, weevils might be shaken down through the grain by the sampling process. Consequently some towers were sampled from the top, the towers being turned upside-down for breaking down at the end of the experiment. No significant difference of results was obtained and it was concluded that the movements noted were real.

The results obtained at ordinary variable room temperature and humidity did not differ from those at constant conditions. A few experiments done in glass towers showed no differences from those in metal towers and excluding light by wrapping black paper around these glass towers also had no effect. Other experiments indicate that sex has no influence on the mobility of weevils nor has the age of the weevil.

A few experiments were carried out using a box of grain which could be split up into a number of 2 in. cubes. The box was built by stacking, on to a base, a number of wooden sections 8 ins. square and 2 ins. deep, having no top or bottom. This box could be filled with grain, and after adding weevils where desired, closed at the top, using a square board which was weighted down. All cracks were sealed with adhesive tape.

The box could be broken down into layers by a metal plate which was slipped between the sections. Each layer was divisible into 2 in. cubes by a metal grille which fitted into the section. In practice it was necessary to insert the grille first before separating the layer, because the metal plate pushed the grain to the back of the box. For the same reason it was necessary to replace the board and weight on the box before separating each layer. A special fitting was made so that the layer could be placed over 16 bottles which received the grain from the cubes when the metal plate was withdrawn.

Use of this apparatus showed that the weevils moved laterally to the sides of the box, and especially to the bottom and corners thus confirming the conclusions drawn from the results of the horizontal towers and from warehouse observations.

TABLE VIII.

Position of weevils in towers containing grain of several moisture contents.

a. 4-day experiment : 3 moisture contents

Individual towers						Totals		Moisture content %
						Position	Moisture content	
A 304	C 164	A 264	B 127	B 301	C 263	Top 1423	A 1102	A 9·9 – 10·9
B 68	B 103	C 157	C 288	A 31	A 50	Middle 702	B 1034	B 14·3 – 15·2
C 214	A 281	B 162	A 172	C 265	B 266	Bottom 1370	C 1351	C 16·8 – 17·7

b. 4-day experiment : 4 moisture contents

Individual towers				Totals		Moisture content %
				Position	Moisture content	
D 166	B 121	A 144	C 174	Top 605	A 484	A 11·9 – 12·0
B 97	C 151	D 180	A 49	Middle 477	B 423	B 13·5 – 13·9
C 153	A 116	B 55	D 212	Middle 536	C 686	C 14·5 – 14·8
A 175	D 242	C 208	B 150	Bottom 775	D 800	D 15·7 – 16·2

c. 14-day experiment : 4 moisture contents

(i) 4 horizontal towers			(ii) 2 sets of 4 vertical towers					
Totals		Moisture content %	Totals					Moisture content %
Position	Moisture content		Position			Moisture content		
				1st set	2nd set	1st set	2nd set	
End 707	A 563	A 11·6	Top	662	748	A 507	572	A 11·9 – 13·2
Middle 337	B 532	B 13·6	Middle	303	196	B 598	597	B 13·4 – 14·3
Middle 345	C 616	C 15·6	Middle	404	234	C 471	395	C 14·2 – 14·8
End 913	D 601	D 17·1	Bottom	1088	994	D 781	608	D 15·3 – 16·1

Oviposition.

It is of interest to know if weevils moving through grain lay eggs as they move. Consequently some of the samples were kept after the weevils had been removed and examined later for a new generation of weevils. These showed clearly that weevils do lay in the grain as they move through it, but as might be expected, oviposition is lower the quicker the movement. Thus in the horizontal towers in which weevils move rapidly from the centre to the ends there is little laying in middle samples. On the other hand, in vertical towers, the movement of adults is made obvious by a gradual shift in the time of the first and maximum emergence of their offspring from the samples taken at points near to and far from the point of insertion. The faster spreading of the pigmy *C. oryzae* also shows obviously in this way (fig. 4).

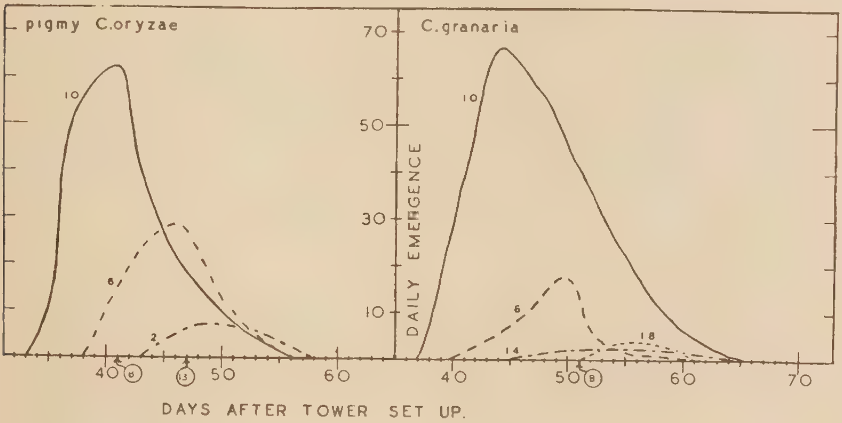

Fig. 4.—Emergence of weevils from the grain used in a long tower (23 sections). The date of the first emergence of weevils from the lowest (B = Bottom) and highest sections producing weevils is shown. Section 18 was the highest reached by *C. granaria*. Data plotted for sections 2, 6, 10, 14 and 18. Weevils were inserted at section 9.

Discussion.

The most important result of these experiments is the unexpected downward movement of weevils in grain. Previous experience has always suggested that they moved upwards but it is based on observation on weevils that have been disturbed, on experiments in very narrow tubes (1 in. diameter, Pielou, unpublished report, 1940), in conditions of heating (Howe, 1943 ; Birch, 1946) or of over-crowding. Also it is clear that given freedom of movement weevils move rapidly to the edges of the bulk and the upper surface is the only one of these normally visible.

It has already been shown in Table III that in nine towers 84 per cent. or more of the weevils were left in the bottom section and in four less than 30 per cent. In most of the experiments detailed in Table V over 60 per cent. of the large weevils were left in the middle sections whereas less than 30 per cent. of the small weevils were generally left there. These two distinct types of result are probably attributable to differences in packing which arose during the transfer of the " set up " tower to the constant temperature room (p. 126). In horizontal towers packing is always loose. The weevils probably could walk on the tower sides in all experiments in which the grain was packed loosely. The marked difference between weevil species of very different sizes suggests that packing is probably the most important factor affecting movement of weevils in bulk grain. In these experiments, therefore, there is more freedom of movement, especially in horizontal towers, than is likely in warehouse conditions. In the warehouse, the packing of the grain gradually tightens as the storage period increases ; in silos the original packing is probably quite tight. Movement of the weevils in the warehouse and silo may be restricted and slow but it is believed that some movement does occur.

It is unfortunate that packing, a comparatively uncontrollable variable, should be so important. Moisture content has some effect in increasing air space and mobility, but whereas an increase of 5 per cent. in moisture content of English wheat to 21 per cent. changes the percentage air space of loosely packed grain from 45 to 48 per cent., tight packing at 21 per cent. moisture content reduces the air space to 39 per cent. (see Jones, 1943).

Accumulation of carbon dioxide in these experiments is unlikely, for in experiments involving dry and damp grain, the damp grain in the middle of the tower was the more popular although carbon dioxide was more likely to occur in these positions (Table VIII b).

In the field, damp grain is most likely to be attractive in ordinary circumstances, although the peculiar preference of laboratory bred weevils for dry grain might be shown by weevils bred under damp conditions in the field.

The ability of weevils to lay eggs at the bottom and in the middle of bulks confirms field observations in warehouses and silos. This is extremely important as adult weevils put at the centre of a large bulk when loaded, or emerging there during storage, are able to lay eggs and produce the larvae which cause heating.

Summary.

The movement of weevils in a mass of grain was investigated using metal pipes 4 ins. in diameter. These grain columns were broken down into samples. It was found that the predominant movement of weevils was towards the bottom.

The major factor affecting movement was tightness of packing but although tight packing restricts movement it does not prevent oviposition. Small insects move more easily in grain than bigger ones and also show a greater tendency to move downwards. Increase of moisture content which increases the amount of airspace also increases the ease of insect movement.

The results of experiments giving the weevils a choice of moisture content were somewhat contradictory. This is partly due to the choice of the weevil being affected by the humidity it experienced prior to the test and partly due to the moisture choice being overridden by the greater attraction of the end of the grain column. On the whole, however, damper grain appears to be preferred to dry grain.

In the field, owing to the tighter packing, movement is likely to be more restricted than in these experiments. The tight packing will prevent much of the downward penetration of weevils observed in these experiments but some such movement may occur on the walls and posts. Where insects are originally placed in the centre of a bulk of grain they will often be immobile but will lay eggs and be liable to cause heating. It is clear that the insects will tend to move toward the edges of the bulk and remain there.

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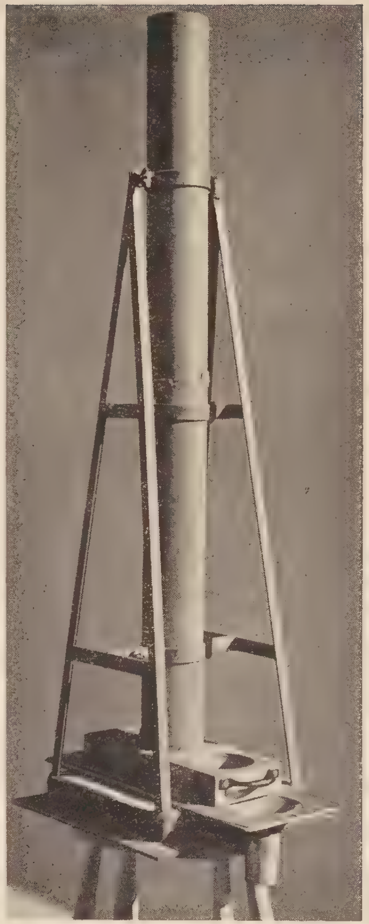

FIG. 1. Photograph of sampling frame with tower in position for

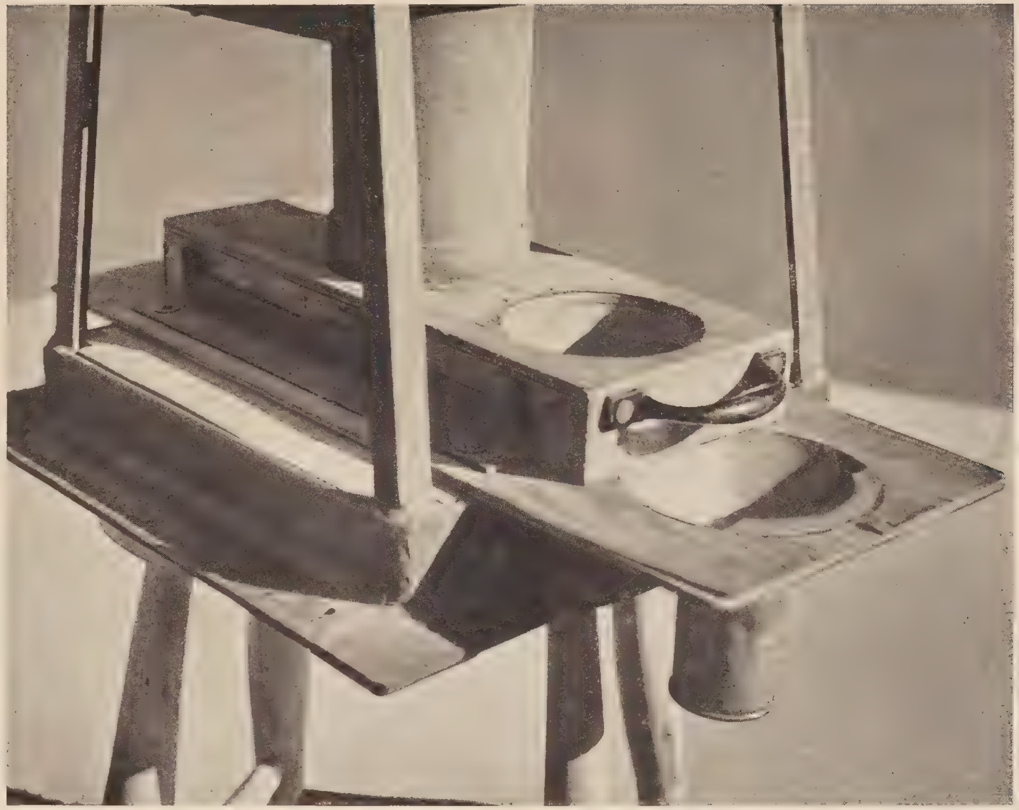

FIG. 2. Photograph of base of sampling frame showing detail.

CONTACT ACTIVITY AND REAL TOXICITY OF SOME IODO-NITROBENZENE COMPOUNDS.*

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Current literature dealing with the contact action of insecticides indicates that a vast amount of experimental work has been carried out with a great variety of chemical compounds. Most of the authors concerned have assessed new or insufficiently known compounds on their toxicity to insects and have not dealt with the more fundamental problems of insecticidal action. When we aim at a deeper understanding of the toxicity of such compounds, then we find many problems still unsolved. One of these problems is the position of substituents, *e.g.* in the benzene nucleus. This has been studied already and some authors tend to favour the para-position over the ortho- and meta-positions, but what makes the p- position favourable is not known. Others have come to different conclusions. The dissimilarity of treatments, and the wide variety of compounds tested, make it difficult to draw conclusions.

An attempt has been made in the work here described to analyse the action of some iodo-benzene compounds in greater detail in order to ascertain whether these substances are promising for insect control, and to what extent and why the position of the iodine atom affects the insecticidal action. The compounds used were p-, m- and o-iodo-nitrobenzene and their dichlorides. Iodine-benzene derivatives have already been tested for insecticidal action by several authors, with varying results. McGovran (1934, 1936) reports a reasonably good action of p-iodo-nitrobenzene against codling moth larvae, *Carpocapsa pomonella* (L.), whereas p-diiodobenzene was less effective. Smith & others (1936, 1938) found p-iodo-nitrobenzene useful against codling-moth larvae and somewhat better than the m- isomer. According to Fink and others (1938) m-iodo-nitrobenzene controls mosquito larvae more satisfactorily than the o- isomer and much better than the p- compound. Bushland (1940) states that m-iodo-nitrobenzene is more effective against screwworms, *Cochliomyia americana* Cush. & Patt., than the o-isomer. Questel & others (1941) found both o- and p-iodo-nitrobenzene useful against corn-borer larvae. Gersdorff & Smith (1940) found p-iodophenol more toxic to goldfish than o-iodophenol and much more so than the m- isomer. The results of the experiments described below, indicate, however, that the position of the iodine atom affects mainly the permeation through the skin. It appears doubtful whether the skin of fish would show permeability phenomena similar to those of the chitinous insect cuticle, as the media are of quite different composition.

Methods.

If the fundamental action of insecticides is to be studied by laboratory methods, it must be borne in mind that the real toxicity, *i.e.* the lethal power of a compound when it has arrived in the tissues of an animal, is the final decisive factor. Consequently suitable conditions must be created to enable this real toxicity to be estimated on a comparable basis. In order to achieve this, animals are injected with the substances concerned and the results expressed in mg. per kg. fresh weight. Such methods, well known to every pharmacologist, have only recently been adopted by entomologists. Detailed information on this subject is given by Dresden (1949).

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* Investigations carried out with the support of the Chilean Iodine Educational Bureau, London.

In the present experiments, cockroaches, *Periplaneta americana* (L.), were injected with the iodine-benzenes. These compounds, being insoluble in water, were dissolved in a small amount of acetone and subsequently emulsified in a physiological salt solution with gum arabic. A microsyringe was used to inject 10–20 cu. mm. emulsion, containing a known quantity of the insecticide, into the abdomen of the cockroach and the dose administered could be expressed in mg./kg. as the insects were weighed before injection. Sixty cockroaches were used for each experiment, and ten others were injected with blank emulsions (mortality in the controls never exceeded 10 per cent). The mortality after six days at 30°C. was calculated by Abbott's formula and the LD50 estimated by the log-probit method of Bliss.

The real toxicity, though extremely important, is not the only factor involved. Under field conditions, the insecticide is applied outside the body and consequently it must pass the cuticle, before its toxic properties come into action. Thus it appears probable that, besides real toxicity, the velocity with which a substance passes through the skin is a decisive factor in contact action, when all other factors are kept constant. Many secondary factors come into play, but if the fundamental action of an insecticide is to be studied, it is necessary to start with the simplest possible conditions. It seems highly important, for instance, to gain some knowledge on the rate of permeation, but unfortunately there are no direct means of obtaining this information. An indirect method is to estimate contact action, which may be considered to be a combination of permeation velocity and real toxicity. A picture of the permeation velocity can then be obtained, provided the real toxicity is known.

In the contact experiments, the method of Krijgsman & Berger (1949, see also Krijgsman, 1947) with *Calandra granaria* (L.) was used. In each of these experiments 50 grain weevils were kept, for a constant time, in a petri dish, the inside of which contained a solid film of the insecticide obtained by evaporation of 2 cc. of a solution of known concentration of the substance in acetone. For each iodo-benzene derivative, 5,000 weevils were used and the LD50 calculated according to Bliss. The LD50 can be expressed in mmg. compound per sq. cm. dish.

Experiments.

I. Para-iodo-nitrobenzene.

(*a*) Contact experiments:

Concentration	Mortality
0·01%	89·0%
0·006%	86·8%
0·004%	58·3%
0·002%	13·5%

LD50=33 ± 1·7 mmg./sq. cm.

(*b*) Injection experiments:

mg./kg. animal injected	Mortality
135	0%
163	33·3%
185	30%
240	50%
255	50%
300	67%
350	100%

From these results the LD50 could be calculated to occur at 270 ± 14 mg./kg. animal.

II. M-iodo-nitrobenzene.

(*a*) Contact experiments:

The contact action of this compound turned out to be extremely low. Grain weevils, walking for three days on a thick solid film, were unaffected. Consequently it was impossible to estimate the contact action of this compound, which lies higher than 1,000 mmg./sq. cm.

(*b*) Injection experiments :

mg. /kg. animal injected	Mortality
360	30·5%
490	67%
600	72%
710	100%

From these results the LD50 could be calculated to occur at 410 ± 20 mg./kg. animal.

III. O-iodo-nitrobenzene.

(*a*) Contact experiments :

Concentration	Mortality
0·045%	99%
0·040%	90%
0·035%	65%
0·030%	30·5%

From these results the LD50 could be calculated to occur at 270 ± 11 mmg./sq. cm.

(*b*) Injection experiments.

mg. /kg. animal injected	Mortality
290	12%
355	50%
405	82%
460	100%

From these results the LD50 could be calculated to occur at 355 ± 20 mg./kg. animal.

IV. P-iodo-nitrobenzene-dichloride.

(*a*) Contact experiments :

Concentration	Mortality
0·02%	64·5%
0·01%	60%
0·005%	58·3%
0·0025%	50·7%

From these results the LD50 could be calculated to occur at 14 ± 0·7 mmg./sq. cm.

(*b*) Injection experiments.

mg. /kg. animal injected	Mortality
140	38%
185	43%
220	60%
270	100%

From these results the LD50 could be calculated to occur at 155 ± 5 mg./kg. animal.

V. M-iodo-nitrobenzene-dichloride.

(*a*) Contact experiments :

The contact action of this compound appeared to be very low. Grain weevils walking for three days on a thick solid film were not killed. Consequently it was impossible to estimate the contact action of this substance. It amounts to more than 1,100 mmg. /sq. cm.

(*b*) Injection experiments:

mg. /kg. animal injected	Mortality
255	44%
280	52%
345	56%
400	84%

From these results the LD50 could be calculated to occur at 310 ± 15 mg./kg. animal.

VI. O-iodo-nitrobenzene-dichloride.

(*a*) Contact experiments:

Concentration	Mortality
0·1%	98%
0·075%	76%
0·050%	18%
0·025%	4%

From these results the LD50 could be calculated to occur at 420 ± 21 mmg./sq. cm.

(*b*) Injection experiments.

mg. /kg. animal injected	Mortality
175	53%
225	77%
285	90%
340	100%

From these results the LD50 could be calculated to occur at 205 ± 5 mg./kg. animal.

Discussion.

The first conclusion that can be drawn from the foregoing experiments is the difference in real toxicity in relation to the position of the iodine atom. P-iodo-nitrobenzene is about 1·5 times more toxic than the corresponding m- and o- compounds (see Table I). Similarly, the p-dichloride compound is more toxic than the corresponding m- and o- derivatives. The chlorine atoms increase the toxicity, for all dichlorides are more toxic than the analogous chlorine-free compounds. These differences in toxicity, though real, are not very striking; greater differences appear when the contact activity is considered. The contact activity, previously mentioned, is a combination of the real toxicity and permeation velocity. As the real toxicity is only slightly altered by the position of the iodine atom, it follows that the big differences in contact action must be caused by considerable variations of the permeation velocity. It must be realised that, in attempting to express the permeation velocity in terms of numbers, the real toxicity for cockroaches is being compared with the contact toxicity for grain weevils. It appears very likely, however, that the real toxicity has general validity, for it has been found (Dresden & Krijgsman, 1948) that the real toxicity of an insecticide is similar for very different animals (insects, frogs, mammals). There is, therefore, no reason why the real toxicity, estimated for cockroaches, would not hold for *Calandra*.

The results are listed in Table I, together with former results with other insecticides (experiments by Dresden, 1949; Dresden & Krijgsman, 1949; Krijgsman, 1949).

The third column of figures refers to the permeation velocity and has been obtained by putting the permeation velocity of γ BHC, quite arbitrarily, at 1,000. The permeation velocity of the other compounds has been calculated relative to this figure. The real toxicity of γ BHC and DDT show the relation 17 : 20; if their permeation velocity had the same magnitude, the contact activities would also

TABLE I.

Substance	LD50 injection mg. /kg.	LD50 contact mmg. /sq. cm.*	Permeation velocity
Benzene hexachloride (pure gamma isomer) ..	17	0·12	1000
Parathion (pure)	1	0·012	590
Tetraethyl pyrophosphate (95% pure) ..	1·5	0·3	35
Velsicol 1068 (technical)	9	2	32
DDT (p,p′)	20	6	24
Dinitro-o-cresol (pure)	85	15	40
Rotenone (pure crist.)	10	very high	<0·08
p-iodo-nitrobenzene	270	33	58
m- ,, ,, ...	410	very high	<3
o- ,, ,, ...	355	270	9
p- ,, ,, dichloride	155	14	75
m- ,, ,, ,, ...	310	very high	<2
o- ,, ,, ,, ...	205	420	3

* The exposure of the test insects was such that any fumigant effect can be discounted for all substances tested, except perhaps parathion, which may have exerted a slight additional fumigant action. This, however, certainly does not affect the essentiality of the listed properties of this insecticide.

be in the same proportion whereas it appears to be 0·12 : 6. Consequently, the permeation velocity of DDT is not 1,000, but only $\frac{20}{17} \times \frac{0\cdot12}{6} \times 1{,}000$ arbitrary units.

In general, the permeation velocity of a compound is :—

$$\frac{\text{LD50 injected compound}}{\text{LD50 injected } \gamma \text{ BHC}} \times \frac{\text{LD50 contact } \gamma \text{ BHC}}{\text{LD50 contact compound}} \times 1{,}000 \text{ units.}$$

The figures obtained in this way only give a rough indication and have no general value. The composition of the cuticle varies considerably in different insects, not ~~the position of the iodine atom than is real toxicity. Whereas the p- position at most~~ possible that permeation velocity calculated from contact action on other insects, would show other sequences. This drawback, however, has to be accepted in the absence of more direct and exact methods.

Although the figures of permeation velocity, listed in the third column of Table I, are only relative, they emphasise the fact that permeation is much more affected by the position of the iodine atom than is real toxicity. Whereas the p- position at most doubles the real toxicity, it increases the permeation velocity by six times and more in the case of the iodo-nitrobenzenes, and by 25 times and more in the case of the dichlorides.*

Other workers have already recorded the greater insecticidal action of p- compounds, but no attempt has ever been made to discriminate between real toxicity and permeation velocity. The present results indicate that the increase in insecticidal action, at least for the compounds tested, is mainly due to an increase in permeation velocity. It would not be surprising if this proved to be a general rule.

For practical purposes, it is obvious that the real toxicity of the iodine compounds is much lower than that of the other insecticides listed. P-iodo-nitrobenzene-dichloride, showing the highest toxicity of the iodine compounds, is still only about half as toxic as dinitro-o-cresol and many times less toxic than the other insecticides. This drawback is offset by high permeation velocity, a velocity that is higher than those of any of the other insecticides tested, except γ BHC and parathion. This high permeation velocity explains its good contact action, which equals that of dinitro-o-cresol and is only a little less than half that of DDT. From these figures it is concluded that p-iodo-nitrobenzene-dichloride is a promising substance. Field experiments and large scale laboratory tests with several insects appear advisable.

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* The small difference in real toxicity of the iodine compounds suggests that their point of action in the tissues is not a very specific one and, when the compounds have arrived in the tissues, their action is not greatly affected by permeability, absorption and the like.

The other iodine compounds do not look very promising. Their real toxicity and permeation velocity do not compare favourably with those of the other insecticides and, in our opinion, further experiments with these compounds are not warranted.

It is obvious from the experimental results discussed in this paper that a discrimination between real toxicity and permeation velocity provides a new approach to a proper understanding of the fundamental action of contact insecticides. Further investigations of a similar nature might lead to a well planned construction of quickly penetrating compounds and to an improvement in compounds that show a high real toxicity but a low permeation velocity. Great care, however, must be exercised in the selection of test objects, for it appears doubtful whether the observed sequence of permeation velocities holds for all insects. Certainly animals with a quite different composition of the skin, *e.g.*, goldfish (Gersdorff & Smith, 1940) must be rejected as test objects.

Summary.

Experiments have been carried out on the insecticidal action of p-, m- and o-iodo-nitrobenzene and their dichlorides. The real toxicity of these substances was estimated with the micro-injection method of Dresden, the contact action with the petri-dish method of Krijgsman & Berger. The real toxicity of the p- compounds appeared to be somewhat higher than that of their isomers. The dichlorides showed a somewhat higher toxicity than the other compounds. The contact action of the p-compounds is much higher than that of their isomers. This is caused by considerable differences in permeation velocity. The position of the iodine atom affects the permeation velocity very considerably but has much less influence on the real toxicity. It is suggested that the enhanced effect of the p- position on the permeation velocity is a general rule.

P-iodo-nitrobenzenedichloride shows such a high permeation velocity that it must be considered as a promising contact insecticide despite the fact that its real toxicity is low as compared with several other modern insecticides.

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PHYSIOLOGICAL INVESTIGATIONS INTO THE HEART FUNCTION OF ARTHROPODS.

THE HEART OF *PERIPLANETA AMERICANA*.*

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The physiological mechanism of the heart of arthropods, chiefly the crustacean heart, has been the subject of many investigations.† These suggest that the automatism of the crustacean heart is not, as in vertebrates, of myogenic but of neurogenic character, but there have been some contradictions amongst the observed results of the action of several drugs and some difference of opinion on their correct interpretation. This may be due to differences in the method of application of the drugs or in the preparation of the subject, as some authors have worked with preparations which are detached from possible regulating centres, and others have worked with subjects in which the action of the drugs on the heart automatism might be complicated by their possible action on other centres.

There is some evidence that the pacemaker of the insect heart is of neurogenic origin (see Krijgsman & others, 1950), but proof is lacking. It is hoped that the detailed experiments with several drugs on the isolated heart described below will contribute to the knowledge of its mechanism and to a better understanding of the action of drugs on arthropods (see also Krijgsman & Krijgsman, 1950).

Methods.

The isolated heart of *Periplaneta americana* (L.), prepared and mounted as described in a previous paper (Krijgsman & others, 1950), was perfused with Yeager's solution (modified Ringer's solution) at 30°C. and observed by means of a binocular microscope. This preparation was entirely free from ventral nerve chord and head ganglia ; consequently the action of the drugs was restricted to the heart and its pacemaker and was not complicated by any possible action on extra-cardial regulating centres. After isolation, the heart was left undisturbed in the experimental vessel for about 30 minutes, in order to enable it to overcome post-operative shock and to regain its normal frequency (80–120 per min.) and type of contraction. The normal Yeager's solution was then replaced by Yeager's solution in which the required drug had been dissolved. Frequency, coordination and type of contraction under permanent perfusion were estimated at short intervals, for one hour or longer.

Experiments were carried out with curare (curadetensine), caffeine, digitalin (digit. pulv. germanic. Merck), strychnine, morphine, apomorphine-HCl, acetylcholine (Roche), nicotine, lobeline (Lobeton hydrochl. crist. Boehringer), pilocarpine, atropine, adrenaline, ergotamine (ergotamin. methansulfonic. Sandoz) and combinations of acetylcholine with tetraethyl pyrophosphate (95 per cent. pure), atropine with tetraethyl pyrophosphate, rotenone (crist.) with acetylcholine, rotenone with tetraethyl pyrophosphate, rotenone with nicotine, rotenone with lobeline, rotenone with pilocarpine, rotenone with digitalin and curare with acetylcholine. The drugs in every case were dissolved in Yeager's solution or in alcohol with subsequent dilution in Yeager's solution and the experiments were repeated several times, in most instances ten times.

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* Experiments carried out in the Netherlands under the auspices of the National Council for Agricultural Research "T.N.O."

† The literature concerned was recently reviewed by Welsh & Schallek (1946).

Experimental.

Curare.

$1{\cdot}5 \times 10^{-4}$ is about threshold value, giving a very slight temporary increase of frequency in most cases. Solutions up to 5×10^{-2} cause a small decrease of frequency but sometimes have no effect at all. Higher solutions were not used. The action of the drug was weak and not clearcut, although much higher concentrations were used than those which show a well marked action in vertebrate animals.

These results are in reasonable accordance with former investigations. Carlson (1907, 1922), for instance, found some accelerating action, followed by inhibition, on the heart of *Limulus*, whereas Davenport (1942) sometimes observed a decrease of frequency of the heart of *Cancer*. The action of the drug, however, was not very pronounced in any of these experiments.

Our experiments lead to the conclusion that the insect heart shows a low susceptibility to curare. In vertebrates, on the other hand, curare exerts a well known blocking action on the motor endplates although not acting in a specific way on the heart.

Caffeine.

10^{-5} is about threshold value, sometimes giving a slight temporary increase of amplitude, in other cases a tendency to incomplete contraction develops.

$2{\cdot}5 \times 10^{-5}$ causes a slight temporary increase of frequency and amplitude in most cases. After a short time, however, heart contractions become incomplete and a lack of coordination develops, as is shown by arrhythmic contractions of different parts of the heart.

5×10^{-5} causes no initial change in frequency, but soon contractions become diastolic, after that a slow decrease of frequency sets in which may lead to a diastolic standstill.

These experiments indicate that the action of caffeine on the insect heart is mainly paralysing, preceded by a small stimulating action at lower concentrations. The results are not in agreement with those of Carlson (*Limulus*), who found only a stimulating action at concentrations of 10^{-4}, whereas our observations showed a paralysing action in lower concentrations. In our view, the action of caffeine on the insect heart is entirely different from its action on that of vertebrates, where it stimulates the myogenic pacemaker of the heart. This different action leads us to believe that the insect heart does not possess a mechanism comparable with the myogenic pacemaker of the vertebrate heart.

Digitalin.

$2{\cdot}5 \times 10^{-7}$ is about threshold value, sometimes causing a doubtful acceleration and a slight increase of amplitude.

5×10^{-7} induces a powerful temporary acceleration of about 100 per cent. and an increase of amplitude. Later, the frequency returns to normal and sometimes a slight irregularity of contractions can be observed.

5×10^{-6} causes an immediate acceleration, frequency going up more than 100 per cent. within a few minutes. The increase of amplitude is also very well marked. After about 30 mins. of powerful and rapid contractions gradually a lack of coordination sets in, though an arrest was never observed in two hours.

5×10^{-5} gives an initial rise in frequency, which soon comes to an end by an abrupt diastolic standstill.

Our experiments show a very well marked action of digitalin on the insect heart; the drug may be considered as strongly stimulating in suitable concentrations. These results do not quite correspond with the action of the drug on the vertebrate heart, where a rise in frequency is lacking. Thus our experiments support the view, that the physiological mechanism of the insect heart differs from that of the vertebrate heart.

Strychnine.

10^{-6}—5×10^{-6} is about threshold value, in most cases giving a slight increase of frequency.

5×10^{-5} causes a moderate but well marked initial rise in frequency of about 30 per cent. but after about 10 mins. the heart tends to incomplete and irregular contractions, alternated by short periods of diastolic standstill.

5×10^{-4} evokes a slight initial decrease of frequency, soon followed by irregular and incomplete contractions, which may lead to shorter or longer periods of diastolic standstill.

This shows that the action of strychnine is diphasic, stimulating the heart in low concentrations and short exposure, and leading to inhibition under longer exposure, or higher concentrations. These results agree to some extent with those of Carlson (*Limulus*), who observed a stimulation by strychnine, and those of Guillebau & Luchsinger (1882, *Astacus*) and Bonnet (1937, *Astacus*), who reported a paralysing action. Apparently not one of these authors observed both phases of the action.

In vertebrates, no specific action of strychnine on the myogenic pacemaker of the heart has been observed; it is confined to the synapses of the central nerve system, which are facilitated by weak solutions or short application and blocked by prolonged action or stronger concentrations. This diphasic action closely resembles the action on the insect heart; it appears very likely, therefore, that the automatic centre of this heart is a nervous structure possessing synapses.

Morphine.

10^{-8} is about threshold value, giving a slight increase of frequency.

5×10^{-8} always gives a steady increase of frequency, amounting to about 90 per cent. after 45 mins.

5×10^{-7} sometimes causes an increase of frequency, but not in all cases. After some time contractions always become slightly incomplete and irregular.

5×10^{-6}—$2{\cdot}5 \times 10^{-5}$. In some cases a slight temporary increase of frequency is induced. After some time, a slow but steady frequency decrease always develops, contractions becoming incomplete and irregular.

5×10^{-5} causes a decrease of frequency, contractions soon becoming incomplete and irregular until finally the heart stands in diastole.

These results point to a well marked diphasic action on the insect heart, small concentrations causing stimulation and bigger concentrations an inhibition of the automatic centre. In vertebrates, no specific action on the myogenic centre of the heart has been reported; on the other hand the diphasic action of this drug on the nerve system is well known, being a stimulation by low and an inhibition by bigger concentrations. The similar behaviour of the pacemaker of the insect heart towards this drug supports the opinion that this pacemaker is a neurogenic structure.

Apomorphine.

5×10^{-7} is about threshold value, sometimes causing a small increase in frequency.

$2{\cdot}5 \times 10^{-6}$ induces a temporary but well marked frequency increase of about 70 per cent., followed by a decrease which may be accompanied by irregular and incomplete contractions, sometimes leading to diastolic arrest.

$2 \cdot 5 \times 10^{-5}$ gives a short temporary rise in frequency, after a few minutes followed by a sharp fall in frequency ; the heart contractions become incomplete and irregular and lead to periods of diastolic arrest.

These experiments show a great resemblance to the action of morphine, both developing a diphasic action, with stimulation followed by inhibition. In vertebrates, the action of apomorphine and morphine are much the same but apomorphine is known to exert a more specific action on the respiratory centre. Our results with apomorphine support those with morphine, and indicate a nervous character of the pacemaker of the insect heart.

Acetylcholine.

$2 \cdot 5 \times 10^{-8}$ is about threshold value, sometimes causing a slight increase in frequency.

$5 \times 10^{-8}-5 \times 10^{-6}$ cause a rapid increase in frequency of about 50 per cent. and an increase in amplitude.

10^{-6} induces a large and immediate frequency rise of about 80 per cent. and increase of amplitude. After some time, however, a slight lack of coordination develops in some cases.

This shows the well pronounced stimulating action of acetylcholine over a wide range of concentrations. It may be stressed that this stimulating action resembles a normal physiological process, as the accelerated heart contracts at high speed, and with increased amplitude, for hours without showing any abnormality. A small irregularity developed after some time only at the highest concentrations and this might indicate the beginning of a blocking action.

These results are in accordance with the action of acetylcholine on most crustacean hearts,* and with the experiments of Hamilton (1939) on *Melanoplus*. Furthermore, they are in full agreement with the action of anticholinesterases on the insect heart (Krijgsman & others, 1950). There is a striking difference in the action of acetylcholine on the vertebrate heart in which it is well known to result in an inhibition of the myogenic pacemaker by supplementing the normal liberation of acetylcholine by the vagus nerve. This again stresses the fact that the arthropod heart does not possess an automatic centre comparable with the myogenic pacemaker of the vertebrate heart. In vertebrates, acetylcholine stimulates all post-ganglionic neurones of the parasympathetic system as well as the preganglionic neurones of the sympathetic system, in other words all cholinergic neurones. As acetylcholine stimulates the pacemaker of the insect heart in a similar manner, it must be concluded that this nervous heart mechanism has cholinergic properties.

Nicotine.

10^{-7} is about threshold value, sometimes causing a slight increase of frequency.

$5 \times 10^{-7}-2 \cdot 5 \times 10^{-6}$ induces an immediate but moderate increase of frequency of about 50 per cent., which is maintained throughout the experiment.

$5 \times 10^{-6}-5 \times 10^{-5}$ causes a similar immediate rise of frequency which is sometimes maintained throughout the experiment ; in other cases, however, the frequency decreases after some time and incomplete contractions set in, alternated by systolic periods.

$2 \cdot 5 \times 10^{-4}$ gives rise to a sharp decrease of frequency, usually leading to a standstill. A subsequent partial restoration of the heart, with irregular and incomplete contractions was observed in some instances.

Nicotine appears from the above experiments to exert a diphasic action, stimulating the insect heart at low concentrations and inhibiting it at high concentrations.

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* There are some Crustacea which are exceptions to this rule (see Baylor, 1942, and Prosser, 1942). It is hoped to consider this problem in a future paper.

These results are in accordance with those of Yeager (1938) with the heart of *Periplaneta* and with the action of this drug on the crustacean heart (Armstrong & others, 1939; Carlson, 1904, 1907, 1922; Bonnet, 1937; Davenport, 1941, 1942; Davenport & others, 1940). Hamilton (1939), however, only observed an inhibitive action on the heart of *Melanoplus*. In vertebrates, nicotine exerts no specific action on the myogenic pacemaker of the heart, but it stimulates cholinergic systems in low, and blocks them in high concentrations. Our experiments, resulting in a similar action on the insect heart, strongly suggest that its mechanism has cholinergic properties, as has already been indicated by the experiments with acetylcholine.

Lobeline.

As the action of nicotine and lobeline are much the same in vertebrates, it might be expected to be the same in the case of the insect heart and this was confirmed by experiment.

10^{-7} is about threshold value, causing a slight increase in frequency.

$2{\cdot}5 \times 10^{-7}$—$2{\cdot}5 \times 10^{-6}$ induces an immediate and permanent rise of frequency of 50–80 per cent.

5×10^{-6}—10^{-5} causes a similar permanent rise of frequency, but later contractions become irregular, systolic periods alternating with incomplete contractions.

5×10^{-4} gives rise to a sudden decrease of frequency, accompanied by phenomena of both incomplete contraction and incomplete relaxation. After a short time contractions stop.

This fully supports the results obtained with nicotine and further corroborates the view that the heart mechanism of this insect has cholinergic properties.

Pilocarpine.

5×10^{-7} is about threshold value, sometimes causing a slight gradual increase in frequency.

10^{-6}—5×10^{-6} induces a sharp immediate and permanent rise in frequency of about 80 per cent.

10^{-5}—5×10^{-5} causes a similar increase of frequency, but sometimes a slight tendency to irregular and incomplete contractions develops after some time.

10^{-4} causes a moderate increase of frequency, often followed by a decrease to subnormal level, accompanied by periods of systolic and of incomplete contractions.

These experiments show a very well marked stimulating action over a wide range of concentrations; only at relatively very high concentrations was there any indication of a blocking action. These results agree very well with those of Garrey (1942), Bain (1929, *Limulus*, *Maia*, *Cancer* and *Carcinus*) and Carlson.

As pilocarpine is a strong stimulator of cholinergic systems in vertebrates, its action on the pacemaker of the insect heart further supports the view that the mechanism of this heart shows cholinergic properties.

Atropine.

10^{-6}—5×10^{-5} is about threshold value, in some cases causing a small increase of frequency.

$2{\cdot}5 \times 10^{-5}$ sometimes induces a small temporary increase in frequency but in other cases this initial increase is lacking. After 10–15 mins. the heart always shows a tendency to incomplete contractions, and a decrease of frequency sets in.

5×10^{-5} causes an immediate lack of coordination, followed by a slow but steady decrease of frequency and incomplete contractions. After some time a very slow irregular beating or a diastolic standstill occurs.

These experiments indicate a small stimulating action at low concentrations and a very well-marked inhibitive action at higher ones. The action of this drug appears to be mainly paralysing. The results obtained by former authors are conflicting. Carlson reported a diphasic action on the heart of *Limulus*, whereas Garrey only observed a stimulating action on this heart. Baylor (1942), Welsh (1942) and Hamilton (1939) only refer to a blocking action on the arthropod heart (*Daphnia*, *Panulirus* and *Melanoplus*), whilst Davenport did not see any action of atropine on the heart of *Cancer*.

In vertebrates, atropine exerts a pronounced paralysing action on cholinergic systems, preceded by a slight stimulating action. As this closely resembles the action on the insect heart, our results with atropine further support the view that the mechanism of this heart has cholinergic properties.

Adrenaline.

10^{-7} is about threshold value, sometimes giving a slight increase in frequency.

$1{\cdot}25-6{\cdot}25 \times 10^{-7}$ causes a moderate increase of frequency, not exceeding 50 per cent.

$1{\cdot}25 \times 10^{-6}$ gives rise to a permanent increase of frequency of about 60 per cent.

$1{\cdot}25 \times 10^{-5}$ causes an initial increase of frequency of about 60 per cent., but after some time frequency decreases again, contractions becoming systolic and irregular.

2×10^{-4} causes an immediate systolic stand.

These experiments clearly indicate a stimulating action ; the tendency to systolic contractions at higher concentrations also point to overstimulation and not to paralysing action. These results are in close accordance with other authors (Bain, 1929 ; Hogben & Hobson, 1924 ; Baylor, 1942 ; Carlson, 1907 ; Davenport & others, 1940 ; Garrey, 1922 ; Welsh, 1939). In vertebrates, the well known action of adrenaline is to accelerate the heart by supporting the sympathetic nerve and by supplementing the normal liberation of adrenergic substances, to support other postganglionic sympathetic neurones. The action of adrenaline on the vertebrate heart compared with that on the arthropod heart suggests that the mechanism of the arthropod heart not only has cholinergic, but also adrenergic properties. If this is right, one may expect ergotamine to exert a paralysing action as this is a well known inhibitor of adrenergic systems.

Ergotamine.

5×10^{-8} is about threshold value, sometimes causing a slight temporary increase in frequency.

$2{\cdot}5 \times 10^{-7}$ may give, in some cases, a slight initial increase in frequency, but, after some time there develops a tendency to incomplete contractions.

$6{\cdot}25 \times 10^{-7}$. Sometimes a slight temporary increase in frequency is induced ; later the heart always develops a lack of coordination and becomes irregular and diastolic.

$1{\cdot}25 \times 10^{-6}$ seldom causes an initial increase in frequency, but always a lack of co-ordination develops, accompanied by irregular and incomplete contractions. Periods of diastolic standstill may occur.

These experiments show clearly that, apart from a slight stimulating action at low concentrations, ergotamine has a paralysing action. The results are in accordance with those of Bain (1929), who reports a paralysing action on the hearts of *Maia*, *Cancer* and *Carcinus*. In vertebrates this drug shows an inhibiting action on adrenergic systems ; consequently the assumption that the heart mechanism of *Periplaneta* has adrenergic properties is supported by the action of ergotamine.

In order to obtain further confirmation of the nature of the automatism of the insect heart, experiments were carried out on the synergism and antagonism of drugs, and these are recorded below.

Acetylcholine and *Tetraethyl Pyrophosphate (TEPP).*

In a former paper (Krijgsman & others, 1950) the strong stimulating action of TEPP on the heart of the cockroach was described. As Richard & Cutkomp (1945) found cholinesterase in nerve tissue of insects, it appeared likely that in the cockroach TEPP acted in a similar manner as anticholinesterases in vertebrates, lengthening the action of normally liberated acetylcholine by counteracting cholinesterase (see also Chadwick & Hill, 1947). Consequently it is highly probable that acetylcholine is a normal neurohumor in the cockroach. This opinion is further supported by our perfusion experiments with acetylcholine (p. 146), which showed an apparently normal physiological action. In order to settle this question definitely, several experiments were carried out with a combination of the two drugs to see whether TEPP supplements the action of acetylcholine.

Perfusion of the heart of the cochroach for 15 mins. with 3×10^{-9} TEPP causes no reaction, as this concentration is below threshold. Subsequent perfusion with 10^{-8} acetylcholine (which is likewise below threshold value) exerts a very well marked rise in frequency and increase of amplitude. The reverse, initial perfusion with acetylcholine followed by TEPP, or simultaneous application, gave similar results. A true synergism of these substances was always observed. These results are in full agreement with those of Davenport (1940, 1941, 1942); Garrey (1922, 1942); Prosser (1942); Roeder & Roeder (1939); Wiersma & Novitski (1942); and Welsh (1939), who all found a sensitisation of the heart to acetylcholine by anticholinesterases in arthropods. Consequently, it may be regarded as certain that acetylcholine is a normal neurohumor in the physiological mechanism of the heart of arthropods, stimulating it as it stimulates cholinergic systems in vertebrates.

Acetylcholine and *Curare.*

According to Davenport (1941, 1942), pretreatment with curare inhibits the action of acetylcholine on the heart of *Cancer*. These experiments were repeated with the cockroach heart, perfusing for 25 mins. with $1{\cdot}5 \times 10^{-4}$ curadetensine and then replacing the curare solution by 5×10^{-7} acetylcholine. Whereas this concentration of acetylcholine induces a ready stimulation by itself (*vide* previous experiments), it did not show any action after administration of curare. The heart maintains its normal activity as if no drug had been applied at all. These experiments are in full agreement with those of Davenport and show a clear antagonism between acetylcholine and curare. In vertebrates, the facilitating action of acetylcholine on motor endplates is negatived by the blocking action of curare. Apparently, similar processes take place in the heart-ganglion of arthropods, probably at the synapses. Although it exerts only very slight action on arthropods when administered alone, curare neutralises the action of acetylcholine. This supports the view that the heart mechanism of insects contains a cholinergic system on which acetylcholine and curare act in an opposite sense.

Atropine and *Tetraethyl Pyrophosphate.*

In order to check the foregoing results, experiments were carried out with atropine and TEPP. If our previous statements are correct (pp. 147–148), then atropine (blocking cholinergic systems) must be antagonistic to TEPP (supporting cholinergic systems) on the insect heart.

In the experiments concerned, a well marked antagonism was in fact observed. Simultaneous perfusion with 2×10^{-6} TEPP (which itself causes a marked acceleration) and $2{\cdot}5 \times 10^{-5}$ atropine shows no acceleration but after some time, a slow delayed atropine action sets in, demonstrated by a steady decrease of frequency and a tendency to incomplete contractions. Pretreatment with TEPP causes a well developed acceleration, which is completely eliminated 15 mins. after perfusion with atropine. These results, indicating an antagonism between the two drugs, are supported by those of Hamilton (1939), Davenport (1942), and Welsh (1939, 1942), who found that the hearts of insects and Crustacea reacted similarly. Garrey (1922, 1942), on the other hand, could not find any antagonistic action between atropine and acetylcholine on the heart of *Limulus*.

According to our results, and the experiments of Hamilton, Davenport and Welsh, atropine and TEPP (or acetylcholine) act in opposition on the arthropod heart and this supports the view that the mechanism of the arthropod heart shows cholinergic properties.

In a previous paper (Krijgsman & others, 1950) it was shown that the well known insecticide rotenone exerts a strong paralysing action on the automatic centre of the insect heart but the point of action remained in doubt. In the light of the knowledge gained on the action of other drugs on the heart of the cockroach, an attempt was made to solve this problem by trying combinations of them with rotenone.

Rotenone and *Acetylcholine.*

Perfusion of the heart with 5×10^{-7} acetylcholine for 25 mins. gives rise to a well marked acceleration of frequency as previously observed. Subsequent perfusion with 2×10^{-8} rotenone counteracts this acceleration, incomplete contractions set in, accompanied by a decrease of frequency which lead to a diastolic standstill. Simultaneous perfusion with 5×10^{-7} acetylcholine and 2×10^{-8} rotenone induces an initial rise in frequency, followed shortly after by the ordinary inhibition caused by rotenone. Perfusion of the preparation with 2×10^{-8} rotenone for 10 mins. caused an inhibiting action but subsequent perfusion with 5×10^{-7} acetylcholine resulted in a partial restoration of the heart, with frequency regaining normal level, but contractions continuing to be incomplete.*

These experiments show that there is a well marked counter-action between rotenone and acetylcholine, but it is not certain whether the point of action is the same.

Rotenone and *Tetraethyl Pyrophosphate.*

Perfusion of the preparation with 3×10^{-7} TEPP for 20 mins. causes a marked increase of frequency. Subsequent perfusion with 10^{-8} rotenone induces a steady decrease of frequency and incomplete contractions. When 3×10^{-7} TEPP is administered again after 40 mins. of rotenone perfusion, a recovery of the heart is observed and a rise of frequency to normal level in about 20 mins. took place. Other experiments, in which the heart was perfused simultaneously with 3×10^{-7} TEPP and 10^{-8} or 2×10^{-8} rotenone, showed a counter-action of the drugs, mostly showing an initial slow rise of frequency (due to TEPP), followed shortly after by a decrease of frequency and diastolic contractions.

From these experiments a similar conclusion can be drawn to that from those with rotenone-acetylcholine. There is a clear counter-action of the drugs, but it is not certain whether the point of action is the same.

Rotenone and TEPP are both powerful insecticides, the former an old well known insecticide (main constituent of derris root), and the latter a post-war insecticide. It is clear from the above that a combination of these insecticides for pest control purposes should be avoided.

Rotenone and *Nicotine.*

The marked stimulating action of weak concentrations of nicotine on the pacemaker of the insect heart has been noted in the previous experiments. Consequently, it appeared of interest to study its action on combination with rotenone.

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* Replacement of rotenone solution by normal Yeager solution never gives a complete recovery.

When the heart was perfused simultaneously with 10^{-8} rotenone and 5×10^{-7} to 5×10^{-6} nicotine there always appeared an initial rise of frequency (caused by nicotine), followed shortly after by a frequency decrease and the other symptoms of rotenone action. A diastolic standstill of the heart, as obtained with rotenone perfusion alone, was seldom observed. In another series of experiments the heart was perfused for 10 mins. with 10^{-8} rotenone, until the inhibiting action had well developed. The rotenone solution was then replaced by 5×10^{-6} nicotine, and this resulted in a partial restoration of heart function, indicated by a slight frequency increase and a more normal type of contraction.

These experiments show a counter-action between rotenone and nicotine, but again, it is not certain whether this phenomenon is a true antagonism.

Rotenone and *Lobeline.*

A number of experiments were carried out with rotenone and lobeline, results similar to those with rotenone-nicotine being expected.

The heart was simultaneously perfused with 10^{-8} rotenone and $2{\cdot}5 \times 10^{-6}$ lobeline. After a short initial rise of frequency (caused by lobeline), a rotenone action set in, causing decrease of frequency and diastolic contractions.

These experiments show a counter-action between rotenone and lobeline similar to that found between rotenone and nicotine.

Rotenone and *Pilocarpine.*

In a number of experiments, the heart was perfused simultaneously with 10^{-8} to $2{\cdot}5 \times 10^{-8}$ rotenone and 10^{-6} pilocarpine. An initial rise of frequency of short duration was always observed, followed by the usual rotenone action. When the heart was pretreated with 10^{-6} pilocarpine for 10 mins., causing the usual symptoms, subsequent perfusion with $2{\cdot}5 \times 10^{-8}$ rotenone gave rise to a delayed rotenone action. When the heart was pre-treated with 10^{-8} rotenone for 15 mins., which caused the usual symptoms, subsequent perfusion with 10^{-6} pilocarpine gave a partial restoration of frequency and size of contractions.

These experiments undoubtedly indicate a counter-action, but again it cannot be said whether rotenone attacks the same point as pilocarpine in an opposite way, or acts on another point of the heart mechanism.

Rotenone and *Digitalin.*

In order to complete the experiments rotenone was tested with digitalin.

In a number of experiments the heart was simultaneously perfused with 10^{-8} rotenone and 10^{-7} to 10^{-4} digitalin. There was not the slightest indication of digitalin symptoms; an initial rise in frequency was always lacking and rotenone acted as if no digitalin had been applied. When the heart was pretreated with 10^{-6} digitalin for 10 mins., causing a well marked frequency rise, subsequent perfusion with rotenone gave rotenone symptoms without any regard to the previous digitalin application. When, on the other hand, the heart was perfused with rotenone for 10 mins., until the symptoms of paralysis were well developed, subsequent perfusion with digitalin, even in strong concentrations, did not influence the rotenone action.

From these experiments the impression was gained that there is no true antagonism between rotenone and digitalin, as the latter cannot influence the action of rotenone, since rotenone always dominates. This suggests different points of action of the drugs.

Discussion.

Previous workers have produced evidence, based on reaction to drugs and histological examination, that the pacemaker of the heart of most Crustacea is a neurogenic mechanism. Little information, however, exists on the heart of insects. Pharmacological experiments have been few, whilst histological examination has brought some evidence that the lateral heart nerves, in which ganglion cells have been found in some cases, act as a pacemaker.

The present experiments have brought ample evidence to support this view. From the action of caffeine, which stimulates the myogenic pacemaker of the vertebrate heart but mainly shows an inhibiting action on the insect heart, it can be concluded that pacemakers in the vertebrate heart differ from those in the insect heart. This conclusion is supported by the action of digitalin which exerts a strong accelerating action on the insect heart, whereas such action is lacking in vertebrates. Furthermore, acetylcholine, which inhibits the myogenic pacemaker of the vertebrate heart, stimulates the insect heart. Nicotine and lobeline, which do not exert a specific action on the myogenic pacemaker of the vertebrate heart, show a well marked diphasic action on the insect heart.

These results all point to a pacemaker mechanism of the insect heart that must be quite different from the myogenic pacemaker of the vertebrate heart. On this assumption, the experiments with strychnine, which acts on the insect heart in the same way as on vertebrate synapses, make it very probable that the pacemaker of the insect heart is a nervous structure in which synaptic transmission plays a part. This conclusion is further supported by the action of morphine and apomorpine, which do not show a specific influence on the myogenic pacemaker of the vertebrate heart, but act on the pacemaker of the insect heart in a similar diphasic manner as on vertebrate nerve centres.

This neurogenic pacemaker of the insect heart is stimulated by acetylcholine, strongly suggesting that the drug is a normal neurohumor of this mechanism. Earlier experiments with tetraethyl pyrophosphate (Krijgsman & others, 1950) support this opinion. Furthermore, it is worthy of note that other specific stimulators of cholinergic systems in vertebrates such as nicotine, lobeline and pilocarpine, act in exactly the same way on the insect heart, whereas atropine, a well-known inhibitor of cholinergic systems, paralyses the insect heart.

Still more of the results point to cholinergic properties of the mechanism of the insect heart. Combinations of acetylcholine and an anticholinesterase (TEPP), which show a synergistic stimulating action on vertebrate cholinergic systems, act in a similar manner on the insect heart. Atropine and an anticholinesterase, on the other hand, have opposing actions on the insect heart, as they have on vertebrate cholinergic systems. Acetylcholine and curare, which act in opposition on the cholinergic motor endplates of vertebrates, also act in opposition on the insect heart.

The heart mechanism of *Periplaneta* has been shown to have cholinergic properties, but the action of adrenaline and ergotamine indicates that the heart mechanism also contains an adrenergic system. Similar results have been obtained with other arthropods. Many workers have observed the stimulating action of acetylcholine as well as adrenaline, and some results point to separate adrenergic and cholinergic systems of the heart mechanism. Bain, for instance, concludes from his experiments that adrenaline and pilocarpine have different points of action on the heart of *Maia*, *Cancer* and *Carcinus*. Furthermore, in his view, atropine does not act in opposition to adrenaline on the heart of these animals.

These indications that separate cholinergic and adrenergic systems exist in the heart mechanism of arthropods require explanation. Wiersma & Novitski (1942) brought some evidence to support their suggestion that the accelerating nerve of the arthropod heart acts by liberation of acetylcholine. This is in accordance with all experiments resulting in acceleration by acetylcholine and cholinergic stimulators. Consequently it appears very likely that the cholinergic system of the mechanism of the arthropod heart is located in the accelerating nerve, liberating acetylcholine, which accelerates the impulse rate from the neurones of the automatic centre. The neurones of this automatic centre, on the other hand, may possess adrenergic properties, liberating adrenergic substances at their endings in the heart muscle. We may therefore visualise acetylcholine and other cholinergic drugs as acting on the neurones of the automatic nerve centre, whereas the point of action of adrenaline and ergotamine lies in the heart muscles.

There is some surprising support for this point of view in the experiments of Carlson (1905a) and Garrey (1922) with the heart of *Limulus*. In this heart the situation is such that a drug can be administered either on the heart ganglion or directly on the heart muscles. Garrey found that acetylcholine stimulates when applied on the heart ganglion but not when applied on the heart muscles. Carlson reports the same for the cholinergic stimulators, nicotine, pilocarpine and eserine, and for the cholinergic inhibitor, atropine. This means that the nerve endings of the heart-ganglion, which form the myoneural junction with the heart muscles, are not cholinergic! On the contrary, they appear to be adrenergic, as shown by Carlson, who found that adrenaline was the only drug to accelerate the heart when applied to the muscles directly.

The present experiments, coupled with the results of other workers, lead to the conviction that the heart mechanism of most arthropods consists of a neurogenic pacemaker with adrenergic properties, giving off impulses with its own rhythm to the heart muscles (see, for instance, Heinbecker, 1936). This spontaneous rhythm is controlled by a cholinergic accelerating nerve.

This mechanism bears some resemblance to the sympathetic nerve system of vertebrates, as the neurones of the accelerating nerve act in the same way as preganglionic cholinergic units, whereas the neurones of the pacemaker act like postganglionic adrenergic units. Nothing is known, however, about the nature of the inhibitor of this system.

It appears improbable that the whole nerve system of arthropods is of a sympathetic nature. Acetylcholine and adrenaline, for instance, exert little specific action on the ventral nerve chord of Crustacea and insects (Prosser, 1942; Roeder & others, 1947; Schallek & Wiersma, 1948). According to the same workers atropine also has no effect. Consequently we have experimental evidence for the opinion, that, as in vertebrates so in arthropods, a certain difference exists between a central nerve system, consisting of ganglia and central nerve cord and an automatic system, which regulates mechanisms such as heart contraction, movements of the intestine, etc.

The action of rotenone is not quite clear. This powerful poison counteracts acetylcholine, tetraethyl pyrophosphate, nicotine, lobeline, pilocarpine and digitalin. Consequently it is quite obvious that a combination of rotenone and tetraethyl pyrophosphate for the control of insect pests is unfavourable. Rotenone may be a strong inhibitor of cholinergic or adrenergic systems, some evidence in support of which was brought by Ambrose & Haag (1936, 1937, 1938) and van Hasselt (1910), who found a low susceptibility of the spinal cord of vertebrates and a much higher susceptibility of the automatic nerve system. More experiments on vertebrate animals, with several combinations of drugs, however, will have to be carried out to establish this.

Summary.

Perfusion of the isolated heart of *Periplaneta americana* with caffeine, digitalin, acetylcholine, nicotine and lobeline shows that the pacemaker of this heart is different from the " myogenic centre " of the vertebrate heart.

The action of strychnine, morphine and apomorphine on this heart preparation affords evidence of the existence of a neurogenic pacemaker.

This pacemaker is stimulated by suitable concentrations of acetylcholine, nicotine, lobeline and pilocarpine, while it is inhibited by atropine. Acetylcholine and tetraethyl pyrophosphate show a synergistic action. Atropine and tetraethyl pyrophosphate, like acetylcholine and curare, show an antagonistic action. These results prove that the neurogenic pacemaker possesses cholinergic properties.

Adrenaline stimulates the insect heart and ergotamine inhibits it, thus suggesting that the insect heart probably also has adrenergic properties.

On the basis of the present work and the results obtained by other investigators, a theory is put forward that the heart mechanism of most arthropods consists of a neurogenic pacemaker with adrenergic properties, controlled by a cholinergic accelerating nerve. This mechanism bears some resemblance to the sympathetic nerve system of vertebrates.

Rotenone strongly counteracts the action of acetylcholine, tetraethyl pyrophosphate, nicotine, lobeline, pilocarpine and digitalin on the insect heart. Its point of action, however, remains obscure at the moment.

The opposing action of rotenone and tetraethyl pyrophosphate is an indication that a combination of these insecticides for pest control is not to be recommended.

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PINK BOLLWORM, *PLATYEDRA GOSSYPIELLA* (SAUND.), AS A PEST OF COTTON AT ZEIDAB, NORTHERN SUDAN.

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A general account of cotton cultivation at Zeidab has already been given (Darling, 1949). Zeidab is a pump irrigation scheme in the Northern Province of the Sudan, lying on the west bank of the Nile about 175 miles north-east of Khartoum. It grows some 5,000 acres annually of long staple American Upland cotton (*Gossypium hirsutum*) or more than 80 per cent. of the entire cotton acreage of the Northern Province.

The climate at Zeidab is hot and dry, having a mean annual temperature of 29·5°C. and a mean annual atmospheric relative humidity of 29 per cent. at 8.00 a.m. Annual rainfall is about 70 mm. and occurs as a few scattered thunder showers in July and August. The winter, which lasts from November to March, is relatively cool with an average daily temperature of 25·6°C., whilst the summer, extending from May to September is hot, having a mean daily temperature of 33·6°C. with midday shade maxima averaging more than 41°C.

The cotton crop is grown in the summer. It is sown in the middle of May and subsequent growth and maturity are so rapid that the main harvest is picked by the middle of September. The plants then yield a small late crop which is picked during October and November and they are finally cut out and destroyed in December.

Pink Bollworm, *Platyedra gossypiella* (Saund.) (Gelechiidae), is perhaps the most important single factor in cotton production at Zeidab. It is the conviction of those with experience of working the scheme that heavy attack is inevitable as the end of the calendar year is approached and sowing in the middle of May was adopted to allow the crop to be harvested before pink bollworm incidence reached its peak in November and December. In spite of May sowing, however, this pest has remained a major factor and is believed to destroy a large part of the potential yield each year (Lord, 1949).

In 1948 an attempt was made to assess the loss due to *P. gossypiella* by examining standard samples of cotton bolls throughout the season and then relating the figures for damage so obtained with the data on crop production. Attention was given to the origin and development of the infestation. The incidence of Egyptian or Spiny Bollworm, *Earias insulana* (Boisd.) (Noctuidae), was also studied to a lesser extent. No other bollworms were seen in the crop.

Estimation of Bollworm Damage in the growing Crop.

One thousand fully grown green bolls were picked from the crop twice a month from the end of July until the middle of November. These were taken from among those bolls which it was estimated would open in the next fortnight ; *i.e.* each 1,000 bolls were representative of the crop that would ripen and be picked before the next thousand were collected. Altogether 8,000 bolls were taken in this way, 1,000 on each of the following dates :—29th July, 14th August, 30th August, 16th September, 30th September, 16th October, 30th October and 16th November. These collections represented the whole crop, the first bolls of which opened about the 10th August. The last watering was given on 2nd November and picking stopped about the end of November.

Surveys of damage were confined to fully grown bolls only. In the northern Sudan, *P. gossypiella* rarely attacks buds, flowers or very young bolls; the loss occurs mainly in the maturing crop and would be shown adequately by the collections described above.

Each collection of 1,000 bolls was made up of four samples, each of 250 bolls, taken from four sites representative of the crop in the south, south centre, north centre and north of the scheme. Bolls were taken at random from each site and successive samples were taken from the same sites.

The day after they were collected, the bolls were opened and examined by trained Sudanese assistants with much experience of this work. The numbers of damaged bolls and of live bollworms per sample were recorded. From 14th August until 30th October the numbers of damaged locules were also recorded, and estimates were made of the numbers of fully grown green bolls and locules per acre to indicate the amount of crop represented by each collection. Information on bollworm damage so obtained is summarised in Table I.

TABLE I.

Incidence of bollworm damage. Zeidab, 1948.

Date	Full grown green crop per acre		Pink bollworm damage			Spiny bollworm damage	
	Bolls	Locules	% of bolls	% of locules	Locules per acre	% of bolls	Bolls per acre
29 July ..	—	—	1·0	—	—	0·9	—
14 Aug. ..	24,290	111,900	4·8	2·0	2,238	1·5	364
30 Aug. ..	59,825	270,900	15·3	3·4	9,320	1·6	957
16 Sept. ..	1,500	6,270	54·7	30·6	1,918	2·1	32
Total ..	85,615	389,070		3·5	13,476	1·6	1,353
30 Sept. ..	9,690	37,740	78·3	51·4	19,400	1·0	97
16 Oct. ..	25,330	95,680	57·3	33·3	31,893	2·5	633
30 Oct. ..	16,306	64,340	49·8	22·8	14,670	8·4	1,370
16 Nov. ..	—	—	48·8	—	—	16·0	—
Total ..	51,326	197,760		33·3	65,963	4·1	2,100

Pink Bollworm was present in small numbers in July. It multiplied rapidly and at the end of September nearly 80 per cent. of the bolls examined showed damage. Most of the attacked bolls at this time contained two or three living larvae. During October and November infestation and damage slowly declined until at the end of the crop about 50 per cent. of the bolls showed damage.

The figures for percentage of bolls damaged by Pink Bollworm suggests that up to 50 per cent. of the potential crop may have been destroyed by this pest. Percentage damage in bolls, however, is not a reliable index to crop loss. Pink Bollworms feed cleanly and usually only damage the seed cotton in the locules in which they develop. Other locules in the same boll, if undamaged, produce normal crop. The percentage of locules damaged by Pink Bollworm is therefore a more accurate indication of the amount of crop destroyed. Assessed on this basis Pink Bollworm damage at Zeidab becomes less serious though obviously still of major importance. During August and September, 13,476 locules were destroyed in an indicated crop of 389,070 locules, giving a loss of 3·5 per cent. During October and November 65,963 locules out of 197,760 were destroyed, or a loss of 33·3 per cent.

Earias insulana was never a major factor in damage to fully grown bolls. Attack was negligible except at the end of the season when it increased to noticeable proportions. Spiny Bollworms are much more destructive in their feeding than are Pink Bollworms. They may invade several bolls in the course of their development and their attack is usually followed by boll-rot and complete loss of the boll. Here, the percentage of bolls damaged is the more reliable indication of the amount of crop destroyed. Even on this basis, however, Spiny Bollworm remains of minor importance compared with Pink Bollworm. During August and September, 1,353 out of 85,165 bolls were destroyed, or 1·6 per cent. In October and November, 2,100 out of 51,326 bolls were lost, or 4·1 per cent.

The inspector in charge at Zeidab, with many years experience of the scheme, regarded the 1948 attack as a normal one.

Estimation of Loss of Yield due to Bollworms.

Table I shows levels of bollworm damage at bi-monthly intervals. By relating these to the timing of the production of the crop they may be integrated into an estimate of loss of yield.

As described in an earlier paper (Darling, 1949) the Zeidab crop matures very quickly. It is sown in the middle of May, flowers in late June and quickly sets a heavy crop of large bolls of up to 40 mm. diameter. This crop is borne on the lower fruiting branches and may be called the " bottom crop ". It ripens and is picked during the last three weeks of August and the first three weeks of September. It produces more than four-fifths of the total yield including all the high grade seed cotton. Table I shows that this bottom crop ripens before bollworms have increased sufficiently to become a serious factor. In 1948, only 5·1 per cent. of the indicated bottom crop was lost through bollworms of which 3·5 per cent. was due to Pink Bollworm and 1·6 per cent. to Spiny Bollworm.

While the bottom crop is developing, growth is arrested. Apparently all the resources of the plants are being used to feed the ripening bolls. In late August growth begins again. New fruiting branches are produced at the tops of the plants and a " top crop " of smaller bolls of 32–35 mm. diameter is set. This ripens during October and November and gives low grade seed cotton amounting to about one fifth of the total yield. This top crop is heavily attacked by bollworms and from Table I a loss of 37·4 per cent. has been calculated. Of this, Pink Bollworm was responsible for 33·3 per cent. and 4·1 per cent. was due to Spiny Bollworm.

Table II gives the weight of the 1948 Zeidab crop classified into grades, and divided into picking periods corresponding to the bottom and top crops.

TABLE II.

Weight of seed cotton in tons. Zeidab crop, 1948.

Time of picking	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Grade 6	Total
Aug.–Sept. .. (Bottom crop)	1,065	570	535	51	1	0	2,022
Oct.–Nov. .. (Top crop)	0	4	82	152	108	83	429
Total ..	1,065	574	417	203	109	83	2,451

From the actual yields set out in Table II and from the percentage losses in yield calculated above from data in Table I, the weights of seed cotton destroyed by bollworms and the potential yields have been calculated and are set out in Table III.

TABLE III.

Losses due to bollworms in tons of seed cotton. Zeidab, 1948.

	Actual yield	Destroyed by pink bollworm	Destroyed by spiny bollworm	Total loss due to both species	Potential yield
Bottom crop	2,022	74	34	108	2,130
Top crop	429	228	28	256	685
Total	2,451	302	62	364	2,815
Total given as per cent. of potential yield ..	87·1%	10·7%	2·2%	12·9%	

Loss of crop was 364 tons of seed cotton or 12·9 per cent. of an estimated potential yield of 2,815 tons. The area under cotton being 5,000 acres, the loss per acre was 163 pounds of seed cotton.

Of the total loss, *P. gossypiella* was responsible for 302 tons or 10·7 per cent. of the potential yield, a loss of 135 pounds of seed cotton per acre. *E. insulana* destroyed 62 tons or 2·2 per cent. of the potential yield, or 28 pounds of seed cotton per acre.

Pink Bollworm is found mainly in the later, top crop. Table II shows that the top crop is low grade seed cotton which fetches a relatively low price. That the top crop is low grade is not entirely due to increased bollworm attack. It is inherently of lower grade than the bottom crop. The financial loss from Pink Bollworm is therefore less than might be expected. The weight of crop estimated to have been destroyed is 10·7 per cent., but because the greater part of this loss was in the low grade top crop the financial loss in 1948 was probably little more than 8 per cent. of the potential total.

The damage caused by Pink Bollworm is undoubtedly serious but in 1948 it was not enough to impair the economic success of the crop. Its complete elimination would not have increased the yield sufficiently to justify expensive control measures, and it is unlikely that chemical control would have paid for itself.

Agricultural Factors influencing the Extent of Pink Bollworm Damage.

(a) *Choice of variety.*

Serious damage at Zeidab is avoided in large measure by the choice of a suitable variety of cotton. Coker-Wilds No. 11 is grown exclusively, the seed being kept pure by periodic importations from the U.S.A. This is a very quick maturing variety, typical of those developed in America to escape the attack of Boll Weevil, *Anthonomus grandis* Boh. (Curculionidae), by producing their crop more rapidly than the weevil can increase to attack it. At Zeidab the crop avoids Pink Bollworm in the same way. Some idea of the speed with which the Zeidab crop ripens is given by comparing it with the production of Sakel type cotton (*Gossypium barbadense*) in the Gezira scheme to the south of Khartoum. At Zeidab four-fifths of the crop is picked within four months of sowing. In the Gezira, little of the crop is harvested within five months of sowing and at least seven months are needed to give four-fifths of the final yield.

(b) *Management.*

Uniformity of crop is as important as rapid maturity. If plants of different ages are grown in the same area, a small initial infestation will bulk up in the bolls on the older plants and will then spread with increased intensity to the young plants when they come into bearing. Any factor or group of factors which delays maturity or produces marked unevenness in the crop will increase pink bollworm damage. Possible factors include :—

1. Successional sowing with a sequence of sowing dates in the same area.
2. Bad agricultural practices such as inefficient watering or poor weed control resulting in checks to growth and an uneven crop.
3. Attack by the Cotton Seedling Worm, *Laphygma exigua* (Hb.) (Noctuidae), in the early days of the crop.

Successional sowing coupled with incorrect agricultural practice at Zeidab usually results in heavy attack by *Laphygma* (Darling, 1949). All three factors then combine to give a late uneven crop providing suitable conditions for serious pink bollworm damage. During the bad *Laphygma* years of 1938–1946 the yields of the scheme dropped markedly below those of years when *Laphygma* was controlled by eliminating successional sowing and by encouraging even, rapid growth. It was of the *Laphygma* years that Lord (1949) was writing when he stated that low yields at Zeidab were caused by bollworm attack. The low yields were most probably due to increased pink bollworm damage consequent upon *Laphygma* attack and other earlier vicissitudes of the crop.

(c) *Sowing date.*

Mention has been made of the conviction that pink bollworm attack increases inevitably towards the end of the calendar year and that by sowing early some of the attack is avoided (p. 157). The results of the present work do nothing to confirm or disprove this belief. It would appear that the development of the infestation is largely conditioned by the state of the crop. The initial infestation may be similarly conditioned so that bollworms will appear in the crop as soon as there are bolls to feed them and infestation will increase with increase in numbers of bolls.

If, however, it could be shown that a considerable initial infestation always appears in the Zeidab area in July and August independently of the state of the growing crop, then the belief would have some justification. Later sowing would mean that the crop would be attacked in July at an earlier stage than at present. A bigger fraction of the growing season would thus be available for increase of infestation and some of the advantage of early maturity would be lost.

No further improvement could, however, be obtained by sowing earlier than at present owing to the risk of increased damage to the ripening bottom crop from the frequent dust storms and the occasional thunder showers of July and August.

Development of Pink Bollworm Infestation in the Crop.

Inspection of the crop in the first half of July failed to show any *P. gossypiella*. It is probable that infestation first appeared in the second half of July, and the collection of 1,000 bolls taken on the 29th of that month contained ten larvae. Precise data are not available but it is probable that the pink bollworm population at the end of July was of the order of 100 to 150 larvae per acre.

From 14th August to 30th October the course of the infestation in the crop was followed by counting the living larvae in the bi-monthly collections of bolls described above and then relating these counts to estimates of the number of full-grown bolls per acre. Populations per acre so calculated are given in Table IV.

TABLE IV.

Pink bollworm incidence in the growing crop. Zeidab, 1948.

Date	Larvae per 1,000 full-grown bolls	Full-grown bolls per acre	Larvae per acre
14 Aug.	45	24,290	1,092
30 Aug.	118	59,825	7,060
16 Sept.	924	1,500	1,390
30 Sept.	1,854	9,690	17,970
16 Oct.	837	25,330	21,190
30 Oct.	625	16,306	10,200
16 Nov.	572	No data available	

By the middle of August the larval population was 1,092 per acre. These larvae were presumably still the first generation produced by the immigrant moths which invaded the crop in the second half of July and early August. The infestation so established increased rapidly and a peak of 21,190 larvae per acre was recorded on 16th October, after which the infestation declined.

The population per acre showed two maxima ; a smaller one at the end of August in the bottom crop, and one three times as big in the middle of October in the top crop. The drop between these maxima corresponds to the hiatus between the two crops when the bollworm population was thought to have been reduced through lack of bolls. Similarly, the fall in numbers after the middle of October is believed to have been due in part to fewer bolls being produced by the ageing plants, and also to an increase in biological control by parasites, notably *Microbracon kirkpatricki* Wlkn. (Braconidae). This small larval parasite was very numerous in the second half of October.

Origin of the Pink Bollworm Infestation.

The old crop is cut out and burnt in late December. The new crop is not suitable for Pink Bollworm until late June. There is a dead season of several months. The adult moth is not known to live longer than a month (Tothill & others, 1940 ; Willcocks, 1916). In July the new crop has a noticeable infestation. The question of the origin of this infestation is of considerable importance. There are four possibilities :—

1. The pest carries over the dead season in the remains of the old crop.
2. The new crop is infested from small populations in alternate host plants in the area.
3. Carry over is by resting larvae (larvae in diapause) in seed cotton stored locally for native cloth manufacture.
4. The initial infestation arrives from outside the area.

The first of these may be discounted. The cutting out of the old crop and the subsequent clean up of plant debris is very thorough indeed. It is unlikely that any infestation persists in this way.

The second possibility is doubtful. Unpublished data on the files of the Sudan Ministry of Agriculture suggest that at Zeidab alternate host plants are not so important in the carry over of Pink Bollworm as they are elsewhere in the Sudan. In the Blue Nile and Khartoum Provinces, bamia (*Hibiscus esculentus*) is attacked by Pink Bollworm to an appreciable extent, but in the Northern Province infestation in this crop has only been recorded when growing near to cotton that was heavily attacked. Other plants in which occasional larvae have been found include *Corchorus olitorius* and *Sida* sp., but the incidence of the pest in these is even less than in *H. esculentus*. In the absence of adequate evidence no definite conclusions may be drawn, but it appears unlikely that alternate host plants play much part in the initial infestation of the crop.

A carry over by resting larvae is a more likely possibility. Though the practice is illegal, some seed cotton is almost certainly stored in the area for native cloth manufacture. This seed cotton presumably contains resting larvae. Observations on the emergence of moths from such larvae in the Zeidab crop have been made over a number of years. At the end of each crop in late November and early December, 10,000 bolls were picked from the plants and caged at Shendi (a town on the Nile some 60 miles south-west of Zeidab and with a very similar climate). Moths emerging from the bolls were caught in a light trap and their number recorded. The results for seven of the years between 1939 and 1949 are given in Table V. During the other three years the work was interrupted by locust campaigns and no results are available.

TABLE V.

Pink bollworm moths from resting larvae in 10,000 bolls picked at the end of the Zeidab crop in late November and early December.

	1939–1940	1940–1941	1941–1942	1942–1943	1943–1944	1947–1948	1948–1949	Average no. of moths from resting larvae
Jan. ..	96	68	285	100	40	203	12	115
Feb. ..	57	39	44	74	96	89	8	58
Mar. ..	71	47	25	20	46	116	18	49
Apr. ..	38	8	47	60	20	79	21	39
May ..	0	0	28	52	16	42	29	24
June ..	0	0	10	13	1	14	0	5
July ..	0	0	1	18	2	7	0	4
Aug. ..	0	0	4	19	1	18	4	7
Sept. ..	0	0	0	2	0	3	1	1
Oct. ..	0	0	0	1	0	0	0	0
Total ..	262	162	444	359	222	571	93	302

Of the yearly average of 302 moths, 285 or 94·4 per cent. emerge during the dead season months of January to May, and only 17 or 5·6 per cent. during the production of the crop. It is doubtful if such a small emergence could produce consistently the infestations observed in the crop. In 1948 on 14th August (Table IV) there were 1,092 first generation larvae per acre, presumably resulting from eggs laid in the last week of July and the first week of August. Allowing that each pair of moths produced 50 effective larvae, this means that during these two weeks there was a population of 44 immigrant moths per acre. In 1948, the 10,000 caged bolls produced 39 moths during June, July and August. These bolls represent about 60 lbs. of seed cotton. In the rather unlikely event of all 39 moths being available during the last week of July and the first week of August, a minimum of 67 lbs. of locally stored seed cotton would be necessary to provide the initial infestation for one acre. At least 150 tons of seed cotton must therefore be stored on the scheme during June, July and August if the initial infestation in 5,000 acres of crop is to be accounted for by local emergence of moths. During these months, in theory, the scheme is completely free of seed cotton and cotton seed. In practice, it is probable that several tons of seed cotton are illegally stored in native villages, but it is very difficult to believe that 150 tons are so concealed. Also such seed cotton is invariably stolen from the crop and since it is worth up to £100 per ton, such thefts would mean a heavy loss to the scheme. The management is most efficient and it is scarcely conceivable that such a loss could pass unnoticed.

On the evidence available it is unlikely that emergences from resting larvae in the Zeidab area can account for all the initial pink bollworm infestation. This evidence is not conclusive however. The results in Table V derive from bolls picked at the end of the crop. It is possible that crop produced earlier in the season may have a bigger population of resting larvae and that a smaller tonnage of such seed cotton would therefore account for the initial infestation ; but any theory involving the illegal storage on the scheme of more than about 15 tons of seed cotton runs into the difficulty of lack of storage space for such a bulky commodity.

There remains the possibility that part of the initial infestation may come from outside the area. Definite evidence is lacking, but Berber, 25 miles north and Shendi-Metemma, 60 miles south of the scheme, are both important centres of native cloth manufacture. It is probable that they and their surrounding villages carry relatively large stocks of seed cotton, some of which may have been stolen from the Gezira scheme where the incidence of resting larvae is higher than is known to occur at Zeidab (see fig. 1). Some of these surrounding villages are within 20 miles of the scheme, and since Williams (1933) has observed pink bollworm moths at 10 miles from the nearest source of infestation, it is possible that some of the initial infestation at Zeidab may be due to moths emerging from seed cotton in villages in the neighbouring areas.

A less likely hypothesis is that part of the initial infestation may come from as far away as Khartoum and Blue Nile Provinces, 175 miles and more to the south. In July and August the south wind blows steadily and in these months in these provinces many moths are produced from resting larvae (see fig. 1, Wad Medani). In addition to illegally stored seed cotton in the villages in the Gezira scheme, the soap factories and oil mills in the Khartoum area carry stock piles totalling several hundreds of tons of untreated cotton seed from the Gezira. This seed is known to be infested with resting larvae. Also in July the summer bamia (*H. esculentus*) crop in these provinces is ripening and is carrying an appreciable pink bollworm population. From all these sources pink bollworm moths are likely to be available in fair quantity in the Nile bank riverain cultivation. It may be possible for some of these moths to drift northwards under pressure from the south wind, and, keeping to the river bank cultivation rather than to the desert, to reach Zeidab.

Factors influencing the Duration of Larval Diapause in Pink Bollworm in the Northern Sudan.

In fig. 1 is shown the seasonal emergence of pink bollworm moths from resting larvae in relation to the water vapour saturation deficit of the atmosphere at Wad Medani, Kadugli, and Zeidab. The saturation deficit values were calculated from the monthly mean 8.00 a.m. relative humidity data and the monthly mean daily temperatures given by Tothill and others (1948) using curves given by Buxton (1931). Data for Atbara, which lies at the northern end of the scheme, were used for Zeidab.

Wad Medani, which represents the Gezira scheme, lies about 110 miles south-east of Khartoum on the Blue Nile. The mean annual rainfall is roughly 400 mm. and the rainy season begins in July and ends in October. The crop, which is Sakel type cotton, is sown in the second half of August and is harvested from January to April before being cut out in May. The emergence figures are quoted from Tothill and others (1948) and are the average of nine years' records from 10,000 bolls caged from each crop. These bolls ripened during February to April when the saturation deficit was rising. Resting larvae so produced remained in their diapause until the steep fall in saturation deficit in June. They then began to pupate and moths emerged during the rains in an abrupt peak in July, August and, to a lesser extent, in September. This peak coincides with the lowest saturation deficit of the year.

Kadugli represents the Nuba Mountains, lying about 400 miles south-south-west of Khartoum, where American Upland cotton is grown on rainfall. The rainy season lasts from June to November and gives an annual average precipitation of about 760 mm. The crop is sown in June and July and is picked from November to January. The emergence figures are taken from the report of the Government Entomologist for 1947–48 (Maxwell-Darling & Darling, 1948). They derive from

25,000 bolls caged in December, 1946, and January, 1947, the emergences being recorded in 1947. These bolls ripened during November and December when the saturation deficit remained constant. The diapause here did not last so well as in the Gezira and moths emerged all through the dry months of February to April. The steep fall in saturation deficit in May and June was accompanied by a small but definite peak of emergence in June and July.

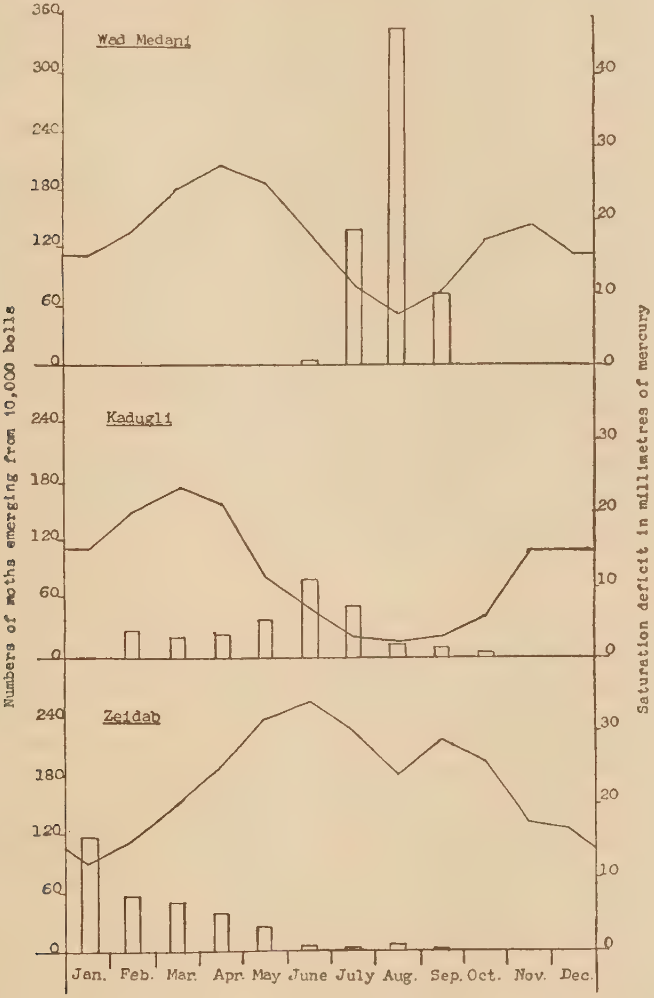

Fig. 1.—Numbers of pink bollworm moths from resting larvae emerging during the year (shown in histograms) plotted against monthly mean water vapour saturation deficit of the atmosphere (curves).

The emergence figures for Zeidab are taken from Table V. Here the bolls ripened during October and November when the saturation deficit was decreasing steadily. The peak emergence of moths occurred in January, when the saturation deficit was at its lowest for the year, and the great majority of the moths had emerged by the end of the dry season in June. The slight fall in saturation deficit in July and August was accompanied by a very small rise in the number of emergences.

Figure 1 shows that in each case the annual peak emergence of moths from resting larvae occurs at or near the annual minimum of the curve of atmospheric water vapour saturation deficit. At Zeidab, where the saturation deficit curve shows two annual minima, a main one in January and a secondary one in August, the emergences also show a main peak in January and a slight second peak in August. These results conform to findings by Squire (1940) that a break in diapause in Pink Bollworm may be brought about by decrease in atmospheric water vapour saturation deficit.

The results in fig. 1 also suggest that the water vapour saturation deficit of the atmosphere during the development of resting larvae may affect the degree of fixity of their diapause. The evaporating power of the air is possibly the most important variable factor determining the water strain of a cotton crop in the northern Sudan. It is possible that a larva surrounded by and feeding on plant tissue that is under ever increasing water strain may be physiologically different from one that is living in a plant under decreasing water strain ; and therefore, that larvae developing in a crop on a rising curve of atmospheric water vapour saturation deficit may differ from those developing on a falling curve. At Wad Medani the larvae develop in a crop on a rising curve and the diapause produced under these conditions is well fixed, lasting unbroken until the curve falls steeply at the onset of the rains. At Kadugli the resting larvae are produced on a level curve of saturation deficit and here the diapause is not so well fixed. Moths begin to emerge immediately and continue to do so all through the dry season. There is, however, a well defined peak emergence coinciding with the fall in saturation deficit in the rains. At Zeidab the resting larvae develop on a falling curve of saturation deficit and here the diapause appears to be poorly fixed. Most of the emergences occur before the end of the dry season. While it is probable that the peak emergence at Zeidab in January is primarily associated with the low atmospheric water vapour saturation deficit of that month, it is also possible that the peak may be partly due to the poor degree of fixity of the diapause making possible such a large emergence so soon after the ripening of the crop.

Summary.

Losses from Pink Bollworm (*Platyedra gossypiella*) and from Spiny Bollworm (*Earias insulana*) were studied in the American Upland cotton crop grown on Zeidab pump irrigation scheme in the Northern Province of the Sudan. In 1948, a year of normal attack, 12·9 per cent. of the estimated potential yield was destroyed by bollworms. Pink Bollworm was responsible for 10·7 per cent. or a loss of 135 pounds of seed cotton per acre. Spiny Bollworm damaged 2·2 per cent. or 28 pounds per acre.

Four-fifths of the total yield is derived from the bottom crop which is harvested before the end of September and consists of high grade seed cotton. The other fifth is produced as a top crop and consists of low grade seed cotton ripening in October and November. Only 3·5 per cent. of the bottom crop is lost on account of Pink Bollworm as against 33·3 per cent. of the top crop. The corresponding figures for Spiny Bollworm are 1·6 per cent. and 4·1 per cent.

Some factors influencing the extent of pink bollworm attack are outlined. The Zeidab crop escapes serious damage through rapid maturity and even growth. Correct agricultural management is of great importance. Attack by *Laphygma exigua* in the seedling stage may result in increased damage by Pink Bollworm in the ripening crop.

The origin of the initial pink bollworm infestation is discussed. Available evidence, which is not conclusive, suggests that local carry over of the dead season may not be sufficient to account for the initial infestations observed in the crop.

Atmospheric water vapour saturation deficit appears to influence the duration of larval diapause in Pink Bollworm in the northern Sudan. Maximum emergence of moths from resting larvae in three areas occurs at or near the lowest saturation deficit of the year. The possibility is discussed that the degree of fixity of the

diapause may be related to the gradient of the saturation deficit curve during the development of the resting larvae in the crop, a rising curve being associated with a well fixed diapause which lasts until the saturation deficit falls, and a falling curve being associated with a poorly fixed diapause which does not last but begins to break at once.

Acknowledgements.

Help from the staff and records of the Sudan Plantations Syndicate at Zeidab, and of the Entomological Section, Research Division, Ministry of Agriculture, Sudan Government, is gratefully acknowledged.

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THE *HAEMAGOGUS* MOSQUITOS OF BRAZIL.*

By Henry W. Kumm & Nelson L. Cerqueira.

(Plates VII & VIII.)

Since 1935 there has been a growing appreciation of the importance of *Haemagogus* mosquitos in the propagation of sylvan yellow fever under natural conditions. This possible relationship was first suggested by Soper (1935, 1936) as a result of epidemiological studies carried out in Brazil and Colombia. Three years later Shannon & others (1938) isolated yellow fever virus from adult *Haemagogus* captured in the State of Rio de Janeiro. Subsequently this finding was confirmed many times in Colombia by Bugher & others (1944). Taylor and Theiler (1948) called attention to the correlation between immunity in primates, the prevalence of *Haemagogus* and the type of forest in which the virus had been found.

In the laboratory, transmission of yellow fever by the bite of *Haemagogus spegazzinii* Brèthes was obtained first by Antunes and Whitman (1937), who called the mosquito *H. janthinomys* Dyar, and later by Waddell (1949). *H. capricornii* Lutz has also been found by Waddell and Kumm (1948) to be a laboratory vector.

Because of their important rôle in the epidemiology of jungle yellow fever, the *Haemagogus* mosquitos of Brazil have been studied intensively in order to ascertain what species exist in this country, what their geographical distribution and relative abundance is, and finally how they can be distinguished one from another. The following report summarises all the information that it has been possible to assemble concerning the prevalence, distribution and taxonomy of the *Haemagogus* of Brazil.

To date only four species of *Haemagogus* have been found in Brazil, and one of them, *H. tropicalis* Cerq. & Ant., in but a single locality. It is evident, therefore, that the *Haemagogus* fauna of this country is less complex than that of Colombia or Central America. For instance, from the tiny Republic of Costa Rica at least five different species of *Haemagogus* were identified by Kumm & others (1940), while no less than eight species were encountered in Colombia by Kumm & others (1946).

Relative Prevalence of the different Species.

Haemagogus mosquitos of one species or another have been obtained in 147 localities in Brazil, *H. spegazzinii* or its subspecies *falco* Kumm, Osorna-Mesa & Boshell-Manrique, having been encountered in 114 places or about three-quarters of the total number surveyed. Not only is *H. spegazzinii* the most widely distributed species in this country, but it is also the most abundant. As shown in Table I, *H. spegazzinii* and *H. s. falco* accounted for approximately 79 per cent. of the total number of male *Haemagogus* identified so far.

In Brazil, adult males of *H. spegazzinii* and *H. s. falco* have only very rarely been captured in nature. The same situation prevails in Colombia.

In order to assemble reliable data dealing with the geographical distribution and relative abundance of *H. capricornii*, *H. spegazzinii* and *H. s. falco*, it is essential, for accurate identification, to examine male genitalia

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* The studies and observations on which this paper is based were conducted with the support and under the auspices of the National Yellow Fever Service of Brazil and of the Yellow Fever Research Service, which was maintained jointly by the Ministry of Education and Health of Brazil and by the International Health Division of The Rockefeller Foundation.

For the correct identification of each adult female *Haemagogus* captured in the forest, it is necessary to permit that mosquito to terminate its blood meal undisturbed and then, in order to obtain oviposition, to transfer it to an individual shell vial, which is provided in the bottom with a pad of moist cotton covered with filter paper. The eggs which are laid must then be matured in a damp state for 10 days or two weeks, after which time they can be submerged in water and satisfactory eclosion will usually take place. The resulting larvae are reared to their pupal and finally to their adult stages. By dissecting the terminalia of one of the males of this group it is possible to ascertain the correct identification of the female originally captured. The information summarised in Table I was obtained by this means.

Whenever possible, collections of adult mosquitos were made in the canopy of the forest, because in Brazil just as in Colombia *H. spegazzinii* and *H. s. falco* are more abundant in the trees than at ground level. Captures were made preferably in the middle of the day, the period of greatest activity among these sylvan species.

TABLE I.

Number of specimens of each species of *Haemagogus* identified and number of localities in which each has been found.

Species of *Haemagogus*	Number of males identified	Per cent. of total represented by each species	Number of localities in which each species has been found
Haemagogus capricornii ..	187	18·3	25
Haemagogus spegazzinii ..	655	64·1	91
Haemagogus spegazzinii falco	155	15·2	23
Haemagogus tropicalis ..	3	0·3	1
Haemagogus uriartei ..	22	2·1	21

The disproportionately small number of male *H. uriartei* Shann. & Del P. identified gives a false impression of the prevalence of that species in Brazil. As it is not necessary to breed out a male in order to identify *H. uriartei*, we have examined only a few male terminalia of that species, which is widely disseminated in the north-eastern states of Brazil.

Geographical Distribution in Brazil of the Species captured.

It is interesting to compare the geographical distribution of the species encountered with altitude and with rainfall. In Colombia, Kumm & others (1946), found seven lowland and only one highland species of *Haemagogus*. The latter, *H. andinus* Osorna-Mesa, was most abundant at altitudes approximating the 2,000 metre contour. In Brazil, however, where most of the country lies at elevations of less than 1,000 metres, the effect of altitude is not so pronounced. Fig. 1 shows that *H. capricornii* and *H. spegazzinii* are not restricted either to the highlands or the to the lowlands of this country. *H. uriartei*, on the other hand, has generally been taken below the 500 metre contour.

The correlation of *Haemagogus* distribution with rainfall (fig. 2) is more evident than its correlation with altitude. *H. uriartei* attains its greatest prevalence in the dry north-eastern states, large areas of which have an annual precipitation of less than 1,000 mm. of rain. This region does not appear to be a favourable one either for *H. capricornii* or for *H. spegazzinii*, the latter having been encountered much more often near the mouth of the Amazon river, where the mean annual rainfall exceeds 2,000 mm. The unusual incidence of *H. s. falco* in the upper reaches of the Amazon basin is probably influenced more by the fact that this zone is situated in the western part of Brazil than by its heavy rainfall. Fig. 2 also indicates that samples of the *Haemagogus* fauna have been obtained in all of the Brazilian States and Territories.

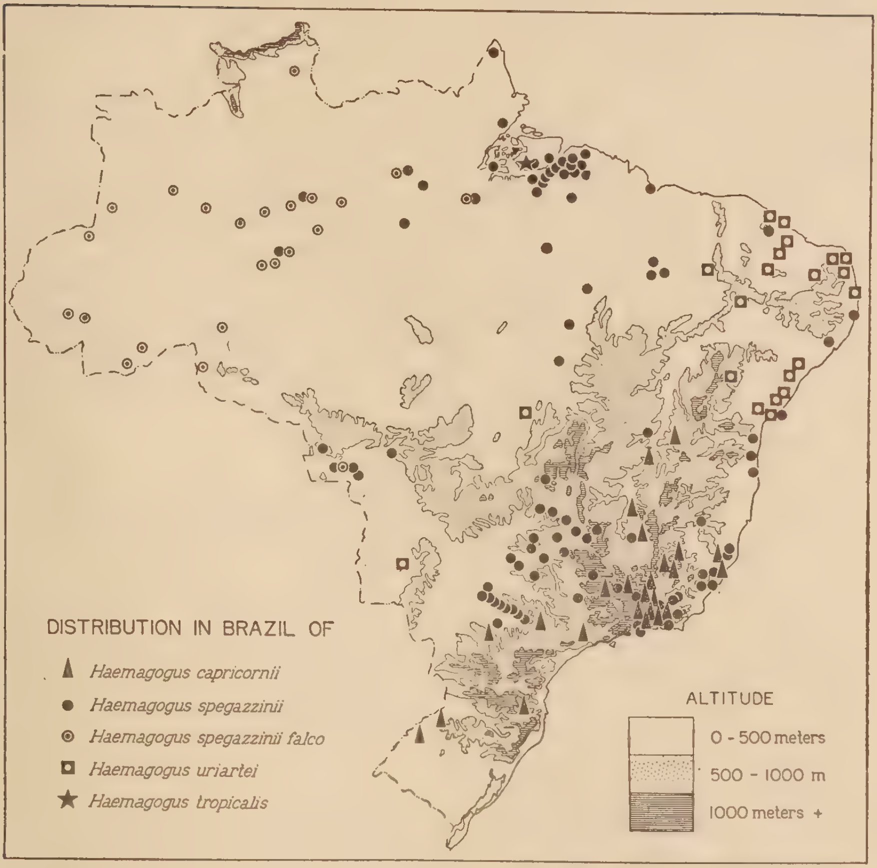

DISTRIBUTION IN BRAZIL OF
▲ Haemagogus capricornii
● Haemagogus spegazzinii
◎ Haemagogus spegazzinii falco
▣ Haemagogus uriartei
★ Haemagogus tropicalis
ALTITUDE
0 - 500 meters
500 - 1000 m
1000 meters +

Fig. 1.

DISTRIBUTION RECORDS OF EACH SPECIES WITH NOTES ON NOMENCLATURE.

Haemagogus spegazzinii Brèthes, Bol. Inst. ent. Pat. veg., Buenos Aires, **1,** p. 39 (1912).

H. janthinomys Dyar, Insec. Inscit. Mens., **9,** p. 112 (1921).
H. spegazzinii Cerqueira, Mem. Inst. Oswaldo Cruz, **39,** p. 10 (1943).
H. spegazzinii Cerqueira & Boshell-Manrique, Proc. ent. Soc. Wash., **48,** p. 191 (1946).

There has been considerable confusion in the past regarding the nomenclature of this species. In a report dealing with certain successful laboratory transmissions carried out by Antunes & Whitman (1937), this species was designated as *H. janthinomys.* Naturally infected specimens were later secured by Shannon, Whitman & Franca (1938) at Bemposta in the State of Rio de Janeiro and were identified as *H. capricornii.* It was not until Boshell-Manrique visited the type locality, namely Ledesma in the Province of Jujuy in northern Argentina, that a correct understanding of the taxonomic position of *H. spegazzinii* was finally obtained (Cerqueira & Boshell-Manrique, 1946).

The 91 localities in Brazil where this species has been encountered are listed below.

States and Territories.	*Localities.*
Amapá	Macapá, Oiapoque.
Alagoas	Atalaia.
Amazonas	Manáus, Manicoré.
Bahia	Canavieiras, Carinhanha, Ilhéus, Maraú, Salvador.
Cearã	Pacoti.
Espírito Santo	Cachoeiro do Itapemirim, Goitacazes, Iconha, Linhares, São Domingos, Vale do Canaã.
Goiaz	Anápolis, Caldas Novas, Catalão, Morrinhos, Pedro Afonso, Porto Nacional.
Maranhão	Carolina, Colinas, Pastos Bons, São João dos Patos, São Luiz.
Mato Grosso	Arado, Mato Grosso, Padre Inácio, Porto Esperidião, Sítio Encantadinho.
Minas Gerais	Além Paraíba, Campina Verde, Curvelo, Frutal, Ituiutaba, Juiz de Fora, Lavras, Leopoldina, Matias Barbosa, Monte Carmelo, Passos, Patos, Patrocínio, Teófilo Otoni, Uberaba, Uberlândia.
Pará	Abaetetuba, Acará, Altamira, Barcarena, Belém, Cametá, Caraparu, Castanhal, Curralinho, Fazenda Pena, Gurupá, Igarapé Mirim, Irituia, Itaituba, Marabá, Mocajuba, Nova Timboteua, Óbidos, Ponta de Pedras, Santarém, São Domingos do Capim, São Miguel do Guamá,Tomé Açu.
Paraná	Sertanópolis.
Pernambuco	Recife.
Rio de Janeiro	Duque de Caxias, Ilha Grande, Mangaratiba, Três Rios.
São Paulo	Assis, Fazenda Bandeirantes, Fernandópolis, Indianópolis, Mirasol, Mococa, Porto Tibiriçá, Presidente Bernardes, Presidente Prudente, Presidente Venceslau, Rancharia, Salto Grande, Santo Anastácio.

Haemagogus spegazzinii falco Kumm, Osorno-Mesa & Boshell-Manrique, Amer. J. Hyg., **43,** p. 13 (1946).

This geographical variety or subspecies of *H. spegazzinii* was first described from Colombia and has also been found in Brazil in that part of the Republic which is near the Colombian frontier. Intermediate types, which appear to be transitions between *H. spegazzinii* and *H. s. falco*, have been taken at Sítio Encantadinho and Sítio Flórida in the State of Mato Grosso, at Guajaramirim in the Territory of Guaporé, at Feijó in the Acre Territory, at Macapá and Oiapoque in the Territory of Amapá, and at Altamira, Óbidos and Santarém in the State of Pará. After a careful study of this situation and after consultation with Dr. Loyd E. Rozeboom, Mr. W. H. W. Komp and Dr. Alan Stone we have concluded that all the forms of *H. spegazzinii* encountered in Brazil belong to one species, which is represented by a continuous series of intergradations. Nevertheless, because the structure of the mesosome of *H. s. falco* is quite distinctive, we have listed below the names of the 23 places where specimens of this subspecies have been taken.

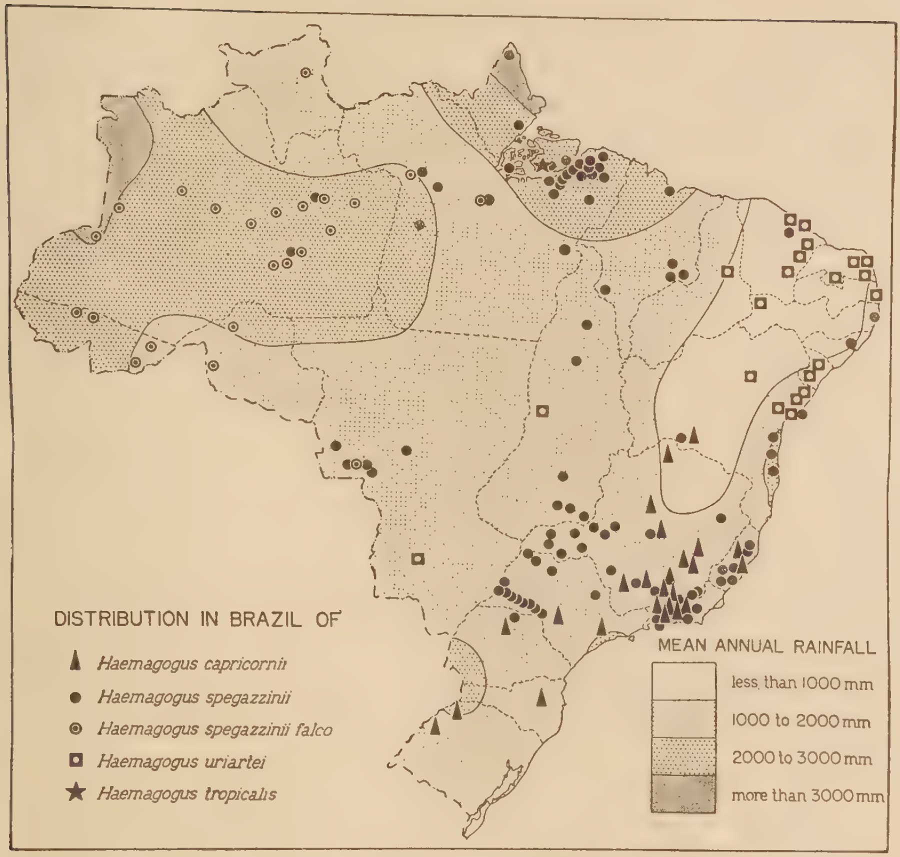

DISTRIBUTION IN BRAZIL OF
▲ *Haemagogus capricornii*
● *Haemagogus spegazzinii*
◎ *Haemagogus spegazzinii falco*
◘ *Haemagogus uriartei*
★ *Haemagogus tropicalis*
MEAN ANNUAL RAINFALL
less than 1000 mm
1000 to 2000 mm
2000 to 3000 mm
more than 3000 mm

Fig. 2.

States and Territories.	*Localities.*
Acre	Feijó, Rio Branco, Tarauacá, Xapuri.
Amazonas ..	Baetas, Benjamin Constant, Borba, Coari, Codajaz, Fonte Boa, Itacoatiara, Limoeiro, Manacapuru, Manáus, Manicoré, São Paulo de Olivença, Tefé.
Guaporé ..	Guajaramirim, Porto Velho.
Mato Grosso ..	Sítio Encantadinho.
Pará	Altamira, Juriti Novo.
Rio Branco ..	Boa Vista.

Haemagogus capricornii Lutz in Bourroul, Mosq. do Braz., p. 66 (1904).
Stegoconops capricorni Lutz, Imp. Med., Rio de J., **13**, p. 83 (1905).
H. capricornii Cerqueira & Lane, Proc. ent. Soc. Wash., **47**, p. 279 (1945).

The distribution of *H. capricornii* in Brazil is more restricted than that of *H. spegazzinii*, the former being largely confined to the south-eastern states. Although originally described by Lutz in 1904, *H. capricornii* was confused for many years with

H. spegazzinii, with *H. janthinomys* and even with *H. equinus*, until Cerqueira and Lane (1945) obtained males from eggs laid by adult females captured in the forest reserve on the Serra da Cantareira near São Paulo, which is the type locality. Specimens of *H. capricornii* have been identified from the following localities in Brazil.

States and Territories.	*Localities.*
Bahia ..	Malhada.
Espírito Santo	Santa Teresa, Vale do Canaã.
Minas Gerais ..	Alfenas, Alvinópolis, Barbacena, Curvelo, Itacarambí, Juiz de Fora, Lavras, Matias Barbosa, Pirapora, Ponte Nova, São Domingos do Prata.
Paraná ..	Apucarana.
Rio de Janeiro	Barra Mansa, Duque de Caxias, Mangaratiba, Paraíba do Sul, Três Rios.
Rio Grande do Sul	Santo Augusto, São Luiz Gonzaga.
Santa Catarina	Ibirama. ..
São Paulo ..	Avaré, Serra da Cantareira.

Haemagogus uriartei Shannon & Del Ponte, Rev. Inst. Bact., Buenos Aires, **5**, p. 68 (1927).

H. uriartei Dyar, Mosq. Amer., p. 135 (1928).
H. uriartei Cerqueira, Mem. Inst. Osw. Cruz, **39**, p. 12 (1943).

This species is quite distinctive in its adult phase because of the greenish copper or greenish gold tinge of its mesonotum, and for that reason it is rarely confused with any of the other *Haemagogus* of Brazil. *H. uriartei* is more abundant in the north-eastern states although a few specimens have been obtained from Goiaz and also from southern Mato Grosso as indicated below.

States and Territories.	*Localities.*
Bahia ..	Alagoinhas, Esplanada, Jacobina, Muritiba, Salvador.
Ceará	Caucaia, Fortaleza, Icó, Limoeiro, Russas.
Goiaz	São Pedro on Bananal Island.
Mato Grosso ..	Aquidauana.
Paraíba ..	João Pessoa.
Pernambuco ..	Araripina.
Piauí	Berlengas.
Rio Grande do Norte.	Acari, Macaíba, Natal, São José do Mipibu.
Sergipe ..	Nossa Senhora das Dores, Simão Dias.

Haemagogus tropicalis Cerqueira & Antunes, Proc. ent. Soc. Wash., **40**, p. 1 (1938).

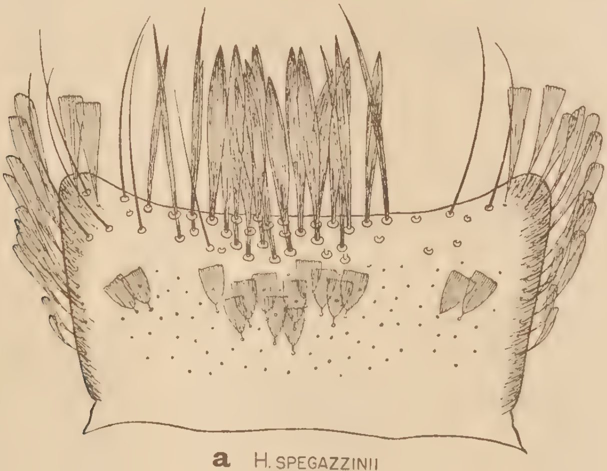
We have only one record of this species from Brazil. Larvae were secured in 1936 at Curralinho, Pará, by the senior author. Although extensive captures have been made since then in many places in the Amazon valley no adults of this species have been obtained, probably because it does not normally attack man.

Keys to Adults, Male Terminalia and Larvae of Brazilian Species of *Haemagogus*.

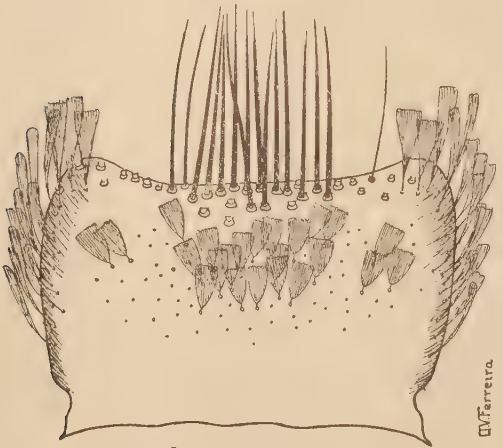
It is not possible to construct adult keys based on external characters or larval keys, which will separate all the Brazilian species of *Haemagogus*. The larvae and females of the three forms with hairy or villose larvae, namely *H. capricornii*, *H. spegazzinii* and *H. s. falco*, are indistinguishable.

At one time it was thought by Cerqueira (1943) that the colour of the scales on the prothoracic lobes of adult females could be used to identify *H. capricornii* and *H. spegazzinii*. In the former the prothoracic lobes were thought to be entirely covered with dark blue scales, while among specimens of *H. spegazzinii* silvery white scales were present along the posterior margin. As the *Haemagogus* survey of Brazil progressed, however, it became apparent that the colouring of the scales on the prothoracic lobes of those two species was a variable character, which could not always be relied upon to distinguish one from the other.

In the following key therefore, only *H. tropicalis* and *H. uriartei* can be identified with certainty. As the characteristic coloration of the mesonotum of *H. uriartei* has not been illustrated previously a special plate (Pl. VII) for that purpose is included. Another plate (Pl. VIII) shows the brilliant hues of *H. spegazzinii*.



a H. SPEGAZZINII



b H. URIARTEI

Fig. 3.—Eighth tergites of *H. spegazzinii* and *H. uriartei*.

Key to Adults.

1. Mesonotum greenish copper or greenish gold in colour ; prothoracic lobes entirely covered with silvery white scales ; abdomen pale blue or greenish blue in colour (Pl. VII) . . . . *H. uriartei*
   Mesonotum greenish blue or blue in colour ; prothoracic lobes covered with blue scales or blue and white scales mixed ; abdomen dark blue or purple in colour (Pl. VIII) 2

2. Male with long palpi ; sternopleural setae absent ; petiole of second wing vein equal to or shorter than the second marginal cell . . . . . . *H. tropicalis*
   Male with short palpi ; one or more sternopleural setae present ; petiole of second wing vein longer than second marginal cell . . . . *H. capricornii*
   H. spegazzinii
   H. s. falco

The only really certain way of identifying all of the species of *Haemagogus* known from Brazil is by their male terminalia. In this country we have found no species with the clasper or spine of the clasper flattened and expanded as occurs in *H. lucifer* (H., D. & K.) and *H. boshelli* Osorna-Mesa from Colombia. The two principal groups of species in Brazil can, however, be separated one from another by the morphology

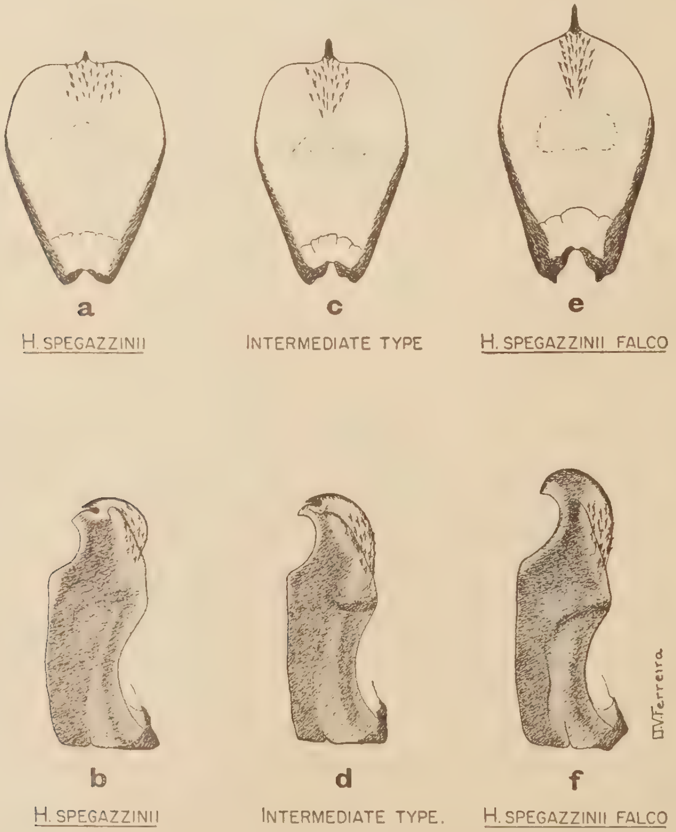


Fig. 4.—Mesosomes of *H. spegazzinii*, intermediate type and *H. s. falco.*

of the scales on the distal margin of the eighth tergite. Among the three proven vectors of jungle yellow fever, namely *H. capricornii*, *H. spegazzinii* and *H. s. falco*, those scales are long, flattened and fusiform, while heavy round setae take the place of those scales on the eighth tergites of *H. tropicalis* and *H. uriartei*. These differences are brought out in fig. 3.

The morphology of the mesosome is an important character for separating *H. capricornii*, *H. spegazzinii* and *H. s. falco*. Numerous spicules are present on the membranous part of the ventral surface of the mesosomes of *H. spegazzinii* and *H. s. falco* as shown in fig. 4. In *H. capricornii*, on the other hand, the mesosome is devoid of spicules, but it has a heavily chitinised diamond-shaped patch below the terminal process, which is most evident when the mesosome is placed in a dorso-ventral position. This is brought out in fig. 5.

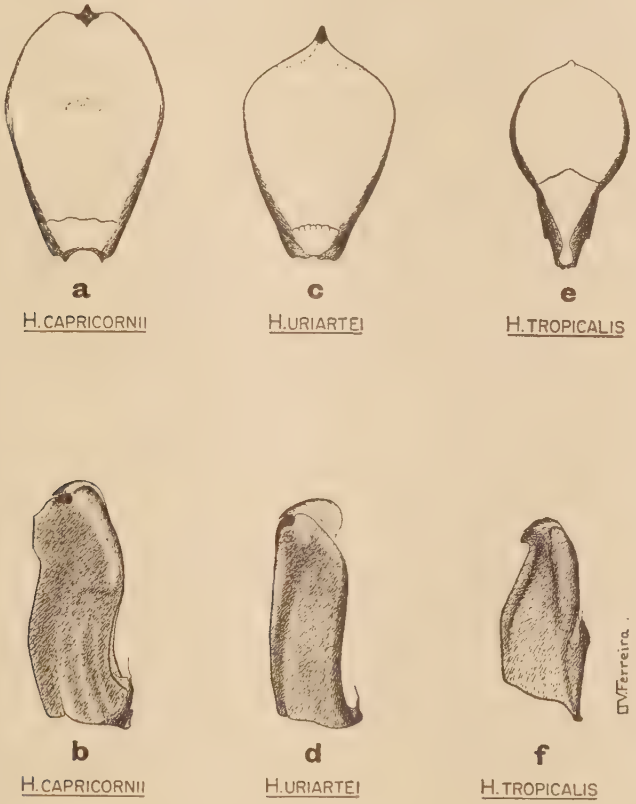
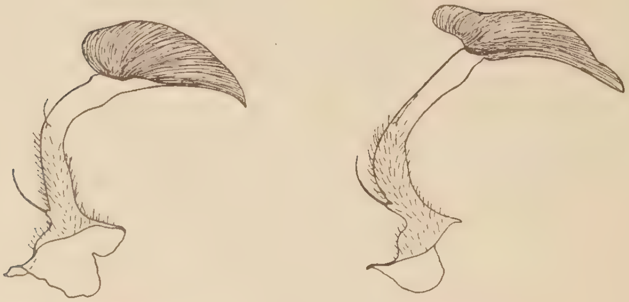
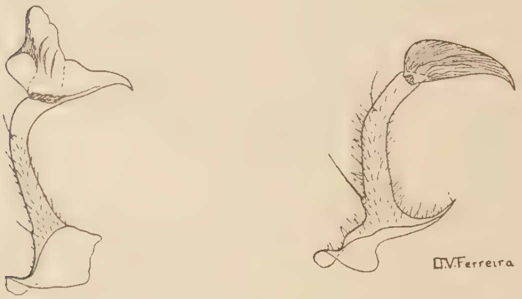


Fig. 5.—Mesosomes of *H. capricornii*, *H. uriartei* and *H. tropicalis*.

The principal difference between *H. spegazzinii* and *H. s. falco* lies in the length of the beaklike terminal process of the mesosome. The exact appearance in those two forms and in an intermediate form are all shown in fig. 4. The shape of the claspette is a convenient character for distinguishing *H. tropicalis* from *H. uriartei*, but not so satisfactory for separating *H. capricornii* from *H. spegazzinii*. The claspettes of all four of the Brazilian species of *Haemagogus* are illustrated in fig. 6.



a H CAPRICORNII **b** H. SPEGAZZINII



c H. TROPICALIS **d** H. URIARTEI

Fig. 6.—Claspettes of Brazilian species of *Haemagogus.*

Among more than a thousand male *Haemagogus* examined during the course of this study, only one gynandromorph has been encountered. It came from São Domingos do Prata in the State of Minas Gerais. That specimen had the external characters, such as plumose antennae, corresponding to a male *Haemagogus*, while the terminalia were those of a female. All other males obtained in that locality were identified as *H. capricornii*, so it was assumed that the gynandromorph belonged to that species also. Since male *H. capricornii* have short palpi, the actual length of the palpi was unaffected by this abnormality.

A key to the male terminalia of the *Haemagogus* of Brazil, based on the characters discussed above, is given below.

Key to Male Terminalia.

1. Eighth tergite with fusiform scales on distal margin (fig. 3, a) ; large tuft of heavy setae arising from a distinct basal lobe on side piece 2
   Eighth tergite with heavy setae instead of scales on distal margin (fig. 3, b); patch of setae on inner lower surface of side piece not arising from a distinct lobe . . 4
2. Ventral surface of mesosome below apex smooth (fig. 5, a) ; beaklike terminal process short and heavily chitinised below (fig. 5, b) . . . *H. capricornii*
   Ventral surface of mesosome with spicules on membranous part below apex (fig. 4, a ; c ; e) ; beaklike terminal process lightly chitinised below (fig. 4, b). . 3
3. Beaklike terminal process of mesosome short (fig. 4, a ; b) . . *H. spegazzinii*
   Beaklike terminal process of mesosome long (fig. 4, e ; f) . . . . *H. s. falco*

4. Filament of claspette large and convoluted (fig. 6, c) ; scales on inner margin of side piece near tip of various shapes, some narrow, some broad and some irregular. *H. tropicalis*

   Filament of claspette small, narrow and with a terminal hook (fig. 6, d) ; scales on inner margin of side piece near tip fairly uniform in size and fusiform in shape. *H. uriartei*

As already pointed out, no satisfactory characters for distinguishing the larvae or even the pupae of *H. capricornii*, *H. spegazzinii* or *H. s. falco* have been found. All three forms have hairy larvae and their comb scales arise from a chitinous plaque. *H. tropicalis* and *H. uriartei* on the other hand can readily be identified both in their larval and in their adult states. A simple key to the larvae follows.

Key to Larvae.

1. Skin of larva villose ; comb scales arising from a chitinous plaque...*H. capricornii*
   H. spegazzinii
   H. s. falco

   Skin of larva glabrous ; comb scales free and not arising from a chitinous plaque . 2
2. Lower anterior pair of dorsal head hairs usually double ; from 17 to 29 comb scales disposed in a patch or in several rows . . . . *H. tropicalis*

   Lower anterior pair of dorsal head hairs always single : from six to ten comb scales arranged in a single row (fig. 7, b) . . . . *H. uriartei*

The Larva of *Haemagogus uriartei* Shannon and Del Ponte.

The larval stage of *H. uriartei* has not previously been described. During this study we have examined 21 larvae and larval skins and these form the basis for fig. 7 and for the following brief delineation.

The skin of the larva of *H. uriartei* is glabrous, a character which distinguishes it from *H. capricornii* and *H. spegazzinii*. The lower anterior pair of dorsal head hairs and the upper posterior pair also are single. There are two tufts of from five to eight small hairs between the anterior dorsal head hairs. The ante-antennal tufts consist of from two to five hairs each, usually three.

On the eighth abdominal segment there is a comb consisting of from six to ten finely barbed scales arranged in a single row. The siphon is three times as long as the width of its base. The number of pecten teeth varies from 16 to 27 with an average of 21. This row of teeth is followed by a pecten tuft of between two and four hairs, generally three. On the lateral surface of the anal segment is a tuft of from three to seven hairs, commonly five. There are four anal gills, the ventral pair being shorter than the dorsal ones. Other details of the morphology of the larva of *H. uriartei* are indicated in fig. 7.

Summary.

Four species of *Haemagogus* have been found in Brazil, the commonest and most widely distributed being *H. spegazzinii*.

Keys and diagrams are given to facilitate the identification of the *Haemagogus* of this country.

Two of the proven vectors of sylvan yellow fever, *H. capricornii* and *H. spegazzinii*, can be distinguished one from another only by their male terminalia.

Acknowledgements.

It would be unjust to terminate this paper without paying special tribute to Arlindo Pinto de Souza and Lério Gomes, two of our faithful co-workers, who have visited numerous remote and isolated areas in this huge country in order to assemble data about the species of *Haemagogus* present in those places. We would also like

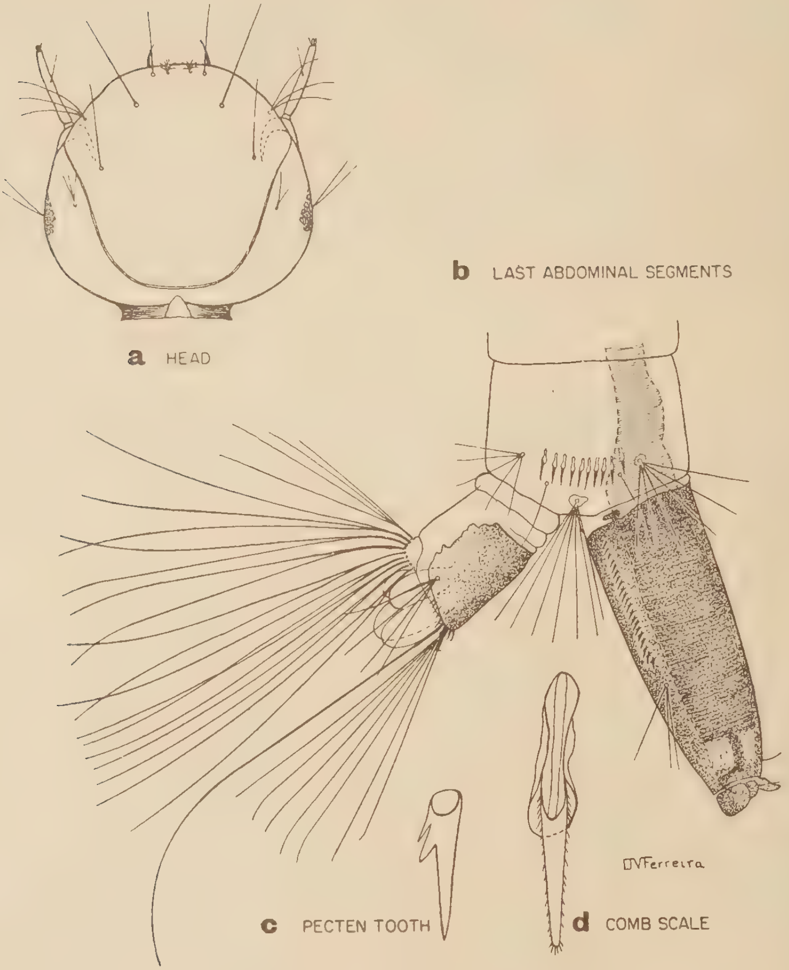

a HEAD
b LAST ABDOMINAL SEGMENTS
c PECTEN TOOTH
d COMB SCALE
DVFerreira

Fig. 7.—Larva of *H. uriartei.*

to express our thanks to John Lane for permitting us to include some of his unpublished information from the State of São Paulo. Many of the diagrams were made by Orlando Ferreira and figs. 1 and 2 were prepared by Claude G. Inman and José Venâncio de Moura.

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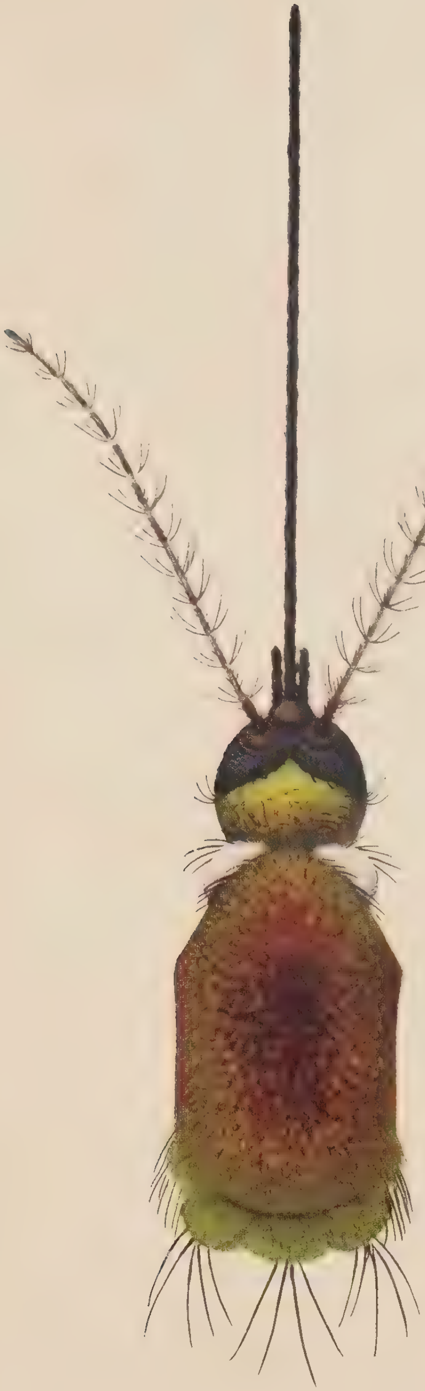
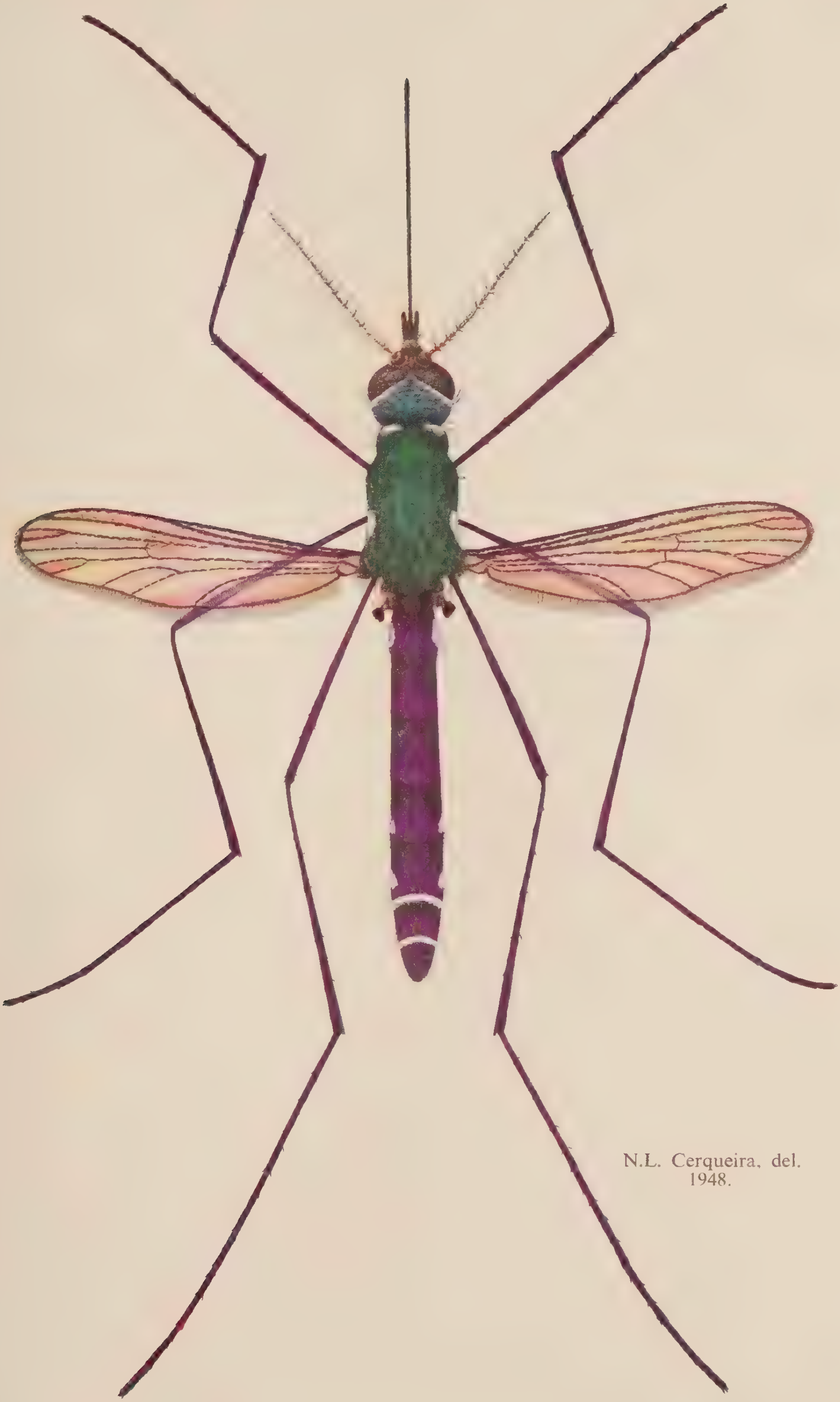


N.L. Cerqueira, del.
1949.

Haemagogus uriartei Shannon & Del Ponte.



N.L. Cerqueira, del.
1948.

Haemagogus spegazzinii Brèthes.

A NEW SPECIES OF *AFRIUS* (HEM., PENTATOMIDAE) PREDACIOUS ON *SCHEMATIZA CORDIAE* BARB., IN MAURITIUS.

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Commonwealth Institute of Entomology, London.

Specimens of the new species here described were received by the Commonwealth Institute of Entomology, London, from Mr. J. R. Williams, Entomologist of the Department of Agriculture, Mauritius, who reported it to be predacious on the Galerucid, *Schematiza cordiae* Barb., which has been introduced there to control the weed, *Cordia macrostachya.*

Afrius williamsi, sp. n. (fig. 1).

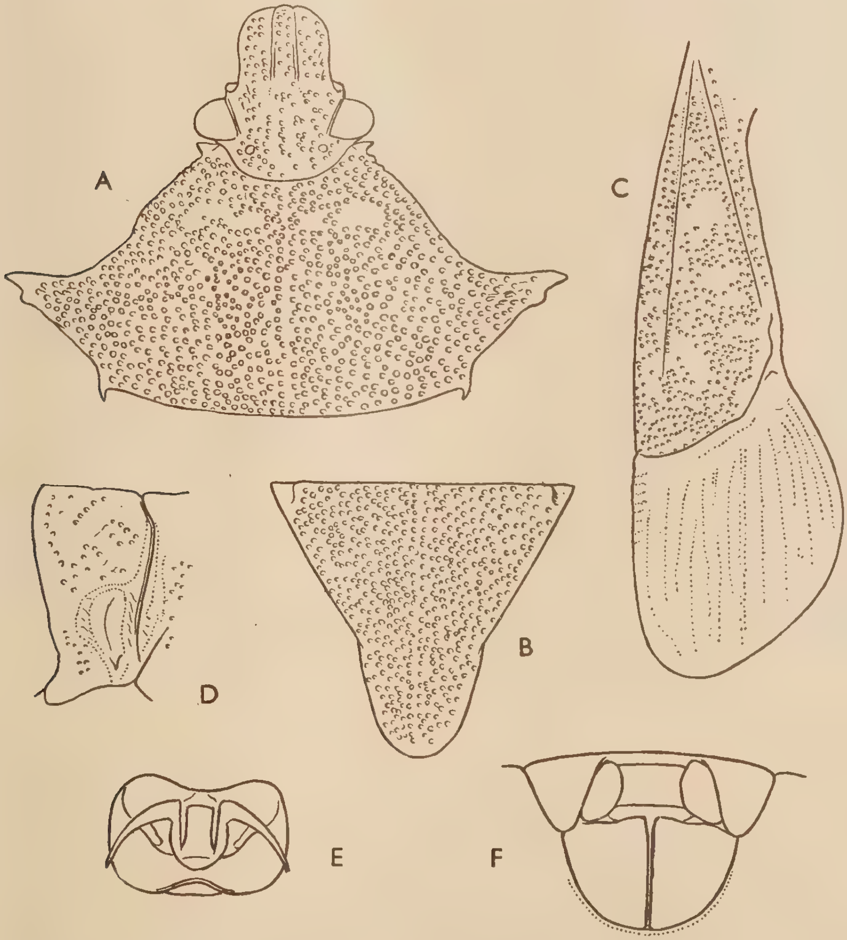


Fig. 1.—*Afrius williamsi*, sp.n. A, head and pronotum, dorsal view; B, scutellum; C, hemelytron; D, ostiole of metathoracic gland; E, pygophore; F, apex of abdomen, ♀.

Segment 1 of antennae brown; segment 2 brown in basal half, piceous or black in apical half; segment 3 piceous, white in basal third; segment 4 dark brown, whitish in basal third. Head, pronotum, propleura and scutellum pale testaceous, strongly suffused with piceous and brown; meso- and metapleura black, except acetabula, an area at upper margin posteriorly of metapleural epimeron pale luteous; prosternum pale luteous with a transverse black stripe, evaporative area around metathoracic gland ostiole greyish; mesosternum piceous with median carina testaceous; metasternum blackish. Abdomen dorsally dark brown with a purplish lustre, connexival segments black, except sub-apically with a suffused reddish-luteous spot and with the lateral margin sub-apically narrowly luteous; ventrally dark brown with segments 6 and 7 laterally with light brown suffusion. Corium pale luteous with faint brownish suffusion and with punctures dark brown; membrane faintly infumate. Femora shining black with pale luteous suffusion; spine pale luteous. Tibiae black; anterior tibiae with a narrow median annulation, median and posterior tibiae with a wide, median annulation pale luteous. Segment 1 of tarsi black with a luteous spot on upper surface; segment 2 brownish suffused with luteous on upper surface; segment 3 brown.

Head dorsally somewhat sparsely punctate; tylus very little shorter than juga which are deflected upwards, lateral margins of juga feebly sinuate; ocellar interspace equal to anteocular at its narrowest part; bucculae feebly punctate and narrowly rounded apically. Anterior lateral angles of pronotum acute, directed outwards, anterior half of lateral margin obscurely serrate near base only, the serrations rounded; spine at postero-lateral angle short, acute, feebly curved. Lateral margins of scutellum feebly widened beyond frenum. Hemelytra extending just beyond apex of abdomen. Tibiae sulcate on outer surface with a feeble rounded elevation at base of sulcus. Male and female genital segments as figured.

Total length: ♂, 8·50—9·0 mm.; ♀, 10·0—10·3 mm.

MAURITIUS: 3 ♂♂ (including the type), 9 ♀♀, 1948-49 (*J. R. Williams*). The type is deposited in the British Museum (Natural History).

This new species, like others of the same genus, varies to some extent in colouration. It also varies in certain structural features, notably the degree of serration of the anterior part of the pronotum laterally and the shape of the projections at the postero-lateral and lateral angles.

The nearest allied species is probably *A. marmoratus* Dall., from which it differs in the shape of the head, the juga hardly extending beyond the tylus, in the shape of the pronotum which, in *marmoratus*, has the anterior half of the lateral margin rounded and strongly serrate, the lateral angles rounded, the spine at postero-lateral angle longer. The scutellum in *marmoratus* has the sides straight and not widened beyond the frenum. The new species also lacks the so-called *macula stridulatoria* present in some species of *Afrius*, including *marmoratus*.

EXPERIMENTS ON THE USE OF DDT FOR PREVENTING BED-BUG INFESTATION OF NEW HOUSES.

By J. R. Busvine, D.Sc., and N. F. Wilson.

Entomology Department, London School of Hygiene and Tropical Medicine.

It has long been realised that when people from old houses in industrial areas are rehoused on new estates, a proportion of them may introduce bed-bugs, *Cimex lectularius* L., with their furniture or luggage. Before the recent War, several progressive local authorities engaged in " slum clearance ", met this difficulty by fumigating the furniture with HCN in special vans during transit.

In recent years, remarkable success has been obtained in eradicating bed-bugs from old property by DDT (Fenton, 1947). This suggests that it might be used to check infestation in new houses by spraying them before occupation. Residual treatment by DDT would be simpler and cheaper than HCN fumigation and, in addition, should provide protection from infestation for several months (*e.g.* supposing the tenants should buy infested second-hand furniture after occupation). A protective treatment would be especially useful with pre-fabricated houses which, by nature of their sectional construction and numerous hollow spaces, could thus provide ideal harbourage for bed-bugs and thus present difficulties in eradication.

A good deal of laboratory data exists on the effectiveness and persistance of DDT films on various surfaces used in traditional and pre-fabricated houses (Barnes, 1945, 1946a). These results are generally satisfactory, except that there is an evident reduction in the effectiveness of DDT applied to recently painted surfaces.

There remains the practical problem of determining what happens to a colony of bed-bugs brought into a DDT-sprayed house on untreated furniture. A full-scale field trial was not considered feasible, for one would need to bring infested furniture, without treatment, into a new house—a condition which would be unacceptable to any housing authority. Eventually it was decided to conduct a trial on a smaller scale, using two huts of prefabricated sections, with rabbits as " tenants " and bug-infested hutches as " furniture ".

Methods Employed.

Two small huts, 3 ft. wide, 8 ft. long and 4 ft. high, were constructed according to the plans shown in fig. 1. The floor was of unpainted plywood, the walls and ceiling of distempered plasterboard. They were assembled in a room at the London School of Hygiene, the temperature of which was maintained at 20°C. (68°F.). The two chambers were sufficiently large to allow a man to get in to make a careful inspection. All crevices were sealed with gummed paper and a tubular glass barrier round the doors prevented escape of bugs liberated inside. The interior surfaces were kept bare except for a number of artificial bug resting places made of glass and cardboard (which, however, the bugs did not use to any great extent).

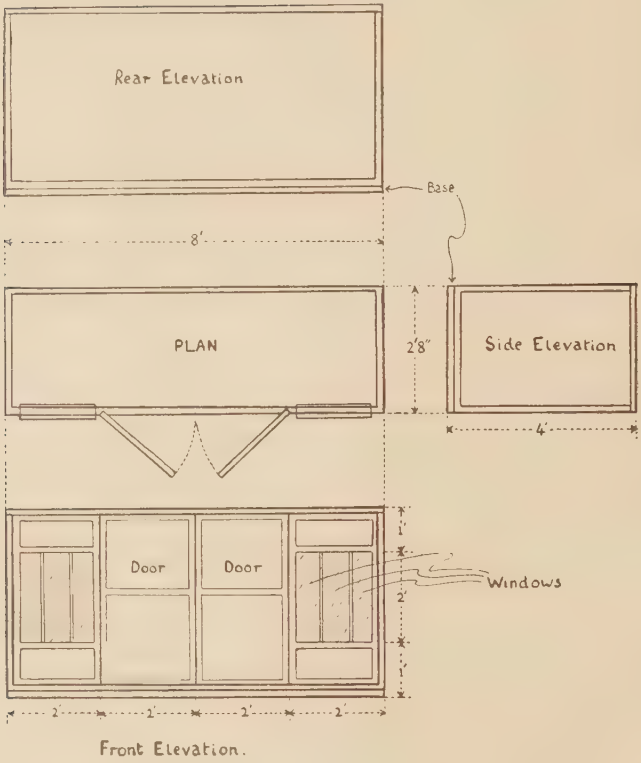

Rear Elevation
Base
8'
PLAN
2'8"
Side Elevation
4'
Door
Door
1'
2'
1'
Windows
2'
2'
2'
2'
Front Elevation.

Fig. 1.—Sketch plan of the experimental huts.

A few days after the huts had been assembled and painted, the interior walls and ceiling of one of them (hut A) were sprayed with 5 per cent. solution of DDT in kerosene to leave a residual deposit of about 170 mg. DDT per square foot. The floor was not treated and was protected from splashes during the application. A week later, a rabbit cage (of the form shown in fig. 2) was placed in each hut. The cages were made of wood, stopped with putty and painted with a flat white paint. Metal grids and trays were supplied to catch the rabbits' droppings, both being removable for inspection. On the outside of each cage was a small glass and cardboard shelter and in this were placed 20 young adult bugs (ten males, ten females) and, two weeks later, another twenty bugs.

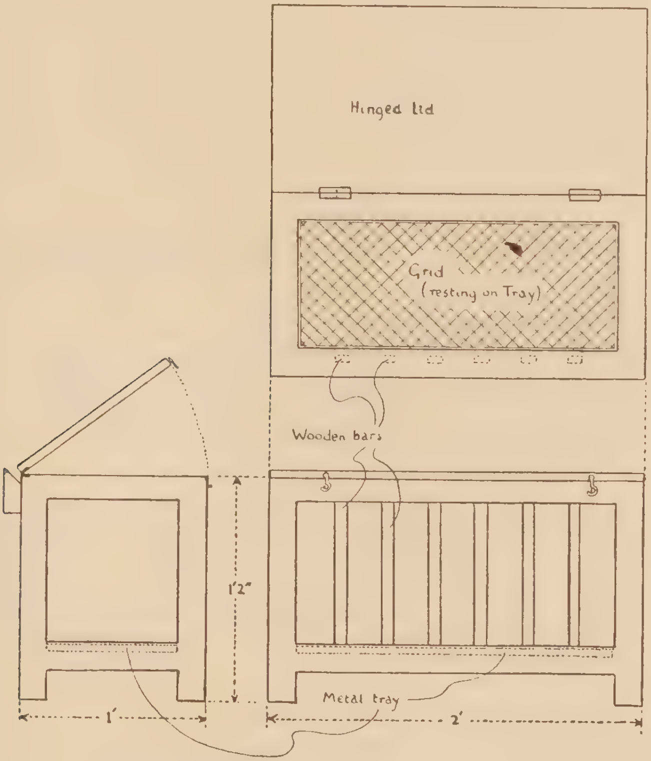


Fig. 2.—Sketch plan of the rabbits' " cage-beds ". *Note.*—Metal tray and grid comprising floor of cage are both removable.

These rabbit cages represented infested beds in a human bedroom and, accordingly, rabbits were placed in them at night only (five nights a week). The experiment was an attempt to parallel the situation where infested beds have been taken into two new houses, one being treated with DDT and one untreated. The course of development of the infestations was watched over the next seven months. At weekly intervals, the whole of the interior of each hut was carefully searched (including the rabbit " cage-bed ") and the numbers of bugs in various stages of development were counted. The interior of each hut was divided into a " grid " of 46 rectangles by lines ruled on the walls and ceiling. This enabled the positions of the bugs observed to be recorded.

Results.

The experiment was begun in November and continued to the end of the following May ; hours of daylight varied, but, as already mentioned, temperature was kept constant throughout.

The observations made in the first 28 weeks of the experiment are given in Table I. In order to present these data more simply, the monthly averages were calculated and are set out in Table II and shown in two diagrams (figs. 3 and 4).

TABLE I.

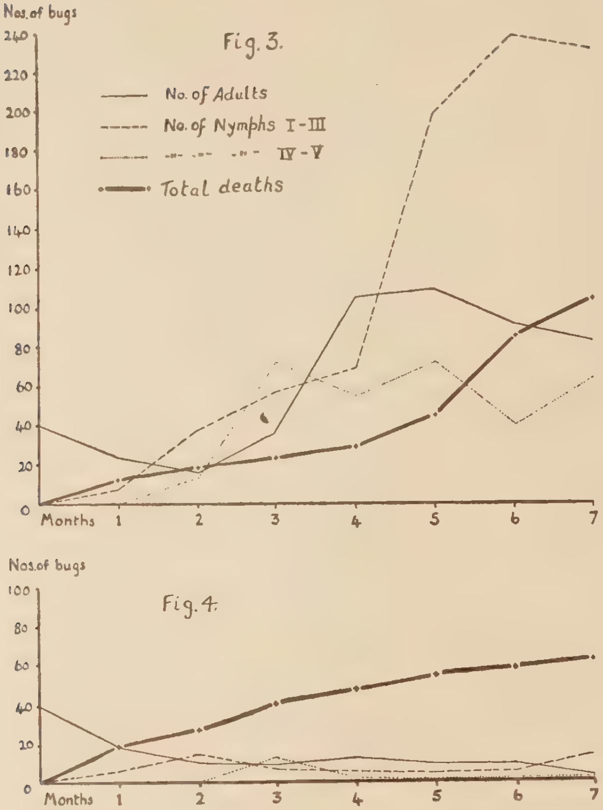
Numbers of bugs observed in experimental huts.

No. of weeks	HUT "A" (treated)					HUT "B" (control)				
	Adults	1, 2, 3 Nymphs	4, 5 Nymphs	Total dead		Adults	1, 2, 3 Nymphs	4, 5 Nymphs	Total dead	
				Adults	Nymphs				Adults	Nymphs
0	20	—	—	—	—	20	—	—	—	—
1	8			10		15			3	
2	6	30		11		9			6	
3	19	10		17		24	10		9	
4	17	0		20		22	14		14	
5	14	24		22	1	22	36		15	
6	9	8		25	1	18	47		16	
7	7	20		25	1	14	48	30	19	
8	4	6	1	26	3	8	19	33	23	
9	6	11	10	31	5	19	59	40	23	
10	6	8	15	32	6	33	103	55	24	
11	7	1	10	33	8	44	53	84	24	
12	8	0	13	33	8	55	19	107	24	
13	13	0	3	35	9	78	22	65	25	2
14	12	0	1	36	9	111	58	59	26	2
15	9	3	0	38	11	124	78	52	30	2
16	6	13	0	41	11	97	114	42	31	2
17	6	6	0	42	11	114	167	48	33	2
18	6	4	2	42	11	103	167	76	38	2
19	5	1	1	43	11	108	209	71	43	2
20	9	0	2	43	11	112	240	94	60	2
21	7	0	1	45	11	111	257	63	74	2
22	6	0	1	46	11	86	229	62	78	3
23	7	8	0	47	11	88	249	77	87	4
24	6	7	0	48	11	74	217	79	89	4
25	3	6	0	49	11	83	250	77	92	4
26	2	10	1	52	12	77	204	69	94	4
27	3	18	1	52	12	91	256	58	101	5
28	2	19	7	52	12	82	221	60	103	5

TABLE II.

Monthly averages of observations.

Month	HUT "A"							HUT "B"						
	Live Bugs				% on "bed"	Dead Bugs		Live Bugs				% on "bed"	Dead Bugs	
	Ad.	N. 1–3	N. 4, 5	Total		Ad.	N.	Ad.	N. 1–3	N. 4, 5	Total		Ad.	N.
0	40	0	0	40	100	0	0	40	0	0	40	100	0	0
1	13	10	0	23	70	18	0	18	6	0	24	68	12	0
2	9	14	0	23	83	25	2	16	38	16	70	83	18	0
3	7	5	12	24	83	32	7	38	59	72	169	68	24	0
4	10	4	1	15	40	37	10	103	68	55	223	64	28	2
5	7	3	1	11	55	43	11	109	198	72	379	55	44	2
6	7	4	1	11	91	47	11	90	238	40	368	63	82	3
7	3	13	2	18	100	51	12	83	233	66	382	57	98	5



Figs. 3 and 4.—Monthly average observations of bugs of different stages :—3, in the untreated hut ; 4, in the DDT-treated hut.

Discussion of Results.

(a) *Effects of the DDT treatment.*

In the DDT-treated house, the population of bugs was reduced and then remained at a low level, without, however, becoming extinct (fig. 4). The colony remained at a level of one or two dozen live bugs up to the end of the seven-month period. The bug population of the untreated hut, on the other hand, rose to a considerable height by the end of six months. Subsequently there is evidence of a slight decline (fig. 3).

The difference between the population trends in the two huts is not easy to explain on the grounds of the observed mortality, which is not greatly different in the two huts at first, and later soars up in the untreated one. There are two probable reasons for the difference.

(i) There is a greater initial death rate in the introduced adults. This initial check is probably important.

(ii) There is probably an important lethal effect of the DDT on very young stages of the bug, the bodies of which are difficult to find and record.

There is some confirmation of this from the data. Thus, at the time of the last observations, about 20 per cent. of the observed deaths in the treated hut were those of nymphs, as compared with only 5 per cent. in the untreated hut.

Translating these results to practical conditions, there is a strong suggestion that a bug colony could just survive in a room treated with DDT provided that the bed (and floor ?) were untreated. The effect of the DDT on wandering bugs, especially the young stages, would, however, severely check population growth and prevent a heavy and widespread infestation.

(b) *Observations on incidence of generations (in the untreated hut).*

The probable composition of the adult bug population, according to generations, is shown in fig. 5. This is based on the following reasoning :—

Supposition	*Observational Basis*
The first generation adults die off fairly uniformly from the beginning of the experiment, about 50% being dead after a month.	Totals of adults alive and dead.
The first generation adults have become rare by the third month or, at least, have stopped oviposition.	Decline in numbers of young nymphs appearing third to fourth months.
The second generation adults begin to appear about the third month, reach a maximum about the fourth month and decline rapidly in fifth to sixth months.	Decline in old nymphs (moulted to adults) and sharp rise of adult curve, followed by decline.
The third generation adults are evidently present (about 50) at the sixth month.	Assuming very few first generation adults remaining, subtract deaths (of second generation) from total adults.
The third generation adults do not seem to have increased by the seventh month.	Total adults curve.

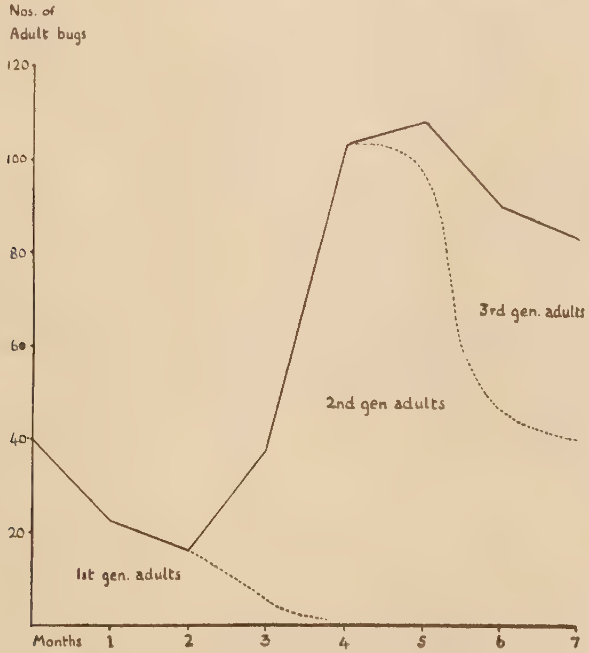


Fig. 5.—Deduced succession of generations in the untreated hut—(see text).

On the basis of the work of Johnson (1942) the total generation (egg to egg) of bed-bugs at 20°C. would be about 88 days ; say three calendar months. This would agree with the appearance of the second generation and third generations of adults.

(c) *Observations on the location of bugs* (*in the untreated hut*).

An impression of the general distribution of the bug population at different stages of the experiment may be gained by the diagrams in fig. 6. These drawings represent an interior view of the untreated hut with the rabbit bed in position. The average numbers of bugs from four weekly bug counts, taken in the first, second, fourth and sixth months, are shown by dots. The dots do not give the exact positions of each bug, of course, but indicate their distribution among the following positions :—

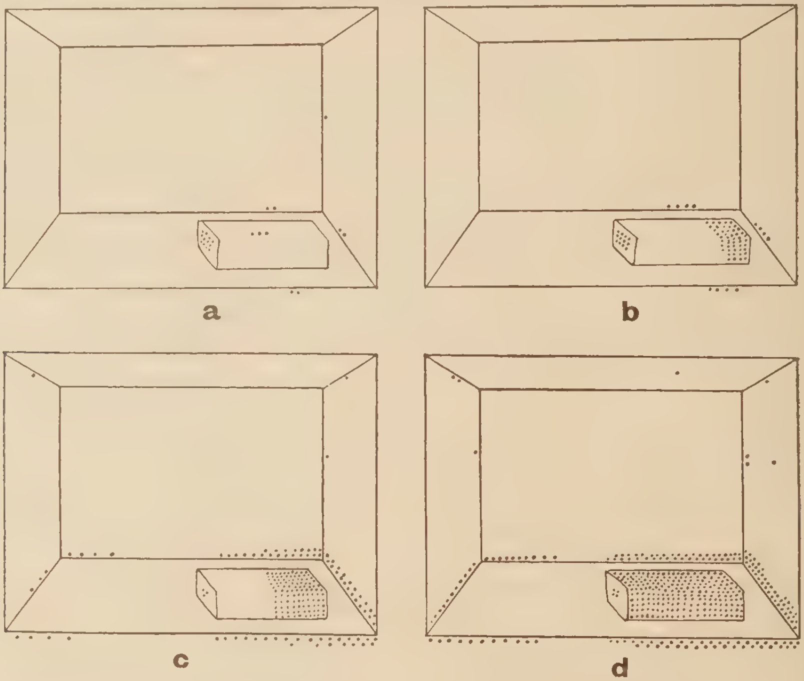


Fig. 6.—Diagrammatic interior view of the untreated hut, with the rabbit " bed " in position, showing the general distribution of bugs in average observations during (1) 1st, (b) 2nd, (c) 4th, (d) 6th months of the experiment. (*N.B.*—The original site of infestation is the visible end of the rabbit " bed ".)

1. Glass shelter on end of rabbit's bed (original site).
2. Remainder of " bed ".
3. Adjacent floor-wall angles.
4. Remote ,, ,,
5. Adjacent wall-wall corners.
6. Remote ,, ,,
7. Adjacent wall-ceiling angles.
8. Remote ,, ,,
9. Open surfaces (floor, wall or ceiling).

("Adjacent" implies the half of the hut in which the rabbit's bed stood, and "remote" the opposite half.)

These diagrams show how little dispersal of bugs took place, even in such a small hut. Most of them remained close to the host. About two-thirds of the observed bugs were usually found about the rabbit's " cage-bed ". Of these, about half were found in the metal tray, about one-third on the woodwork and the remainder on the wire grid covering the tray.

The bugs which wandered away from the " cage-bed " were sometimes observed on the open surfaces, but more usually in the angles between floor and wall or the corners between walls or between walls and ceiling. Sometimes solitary bugs were observed, but there was a tendency for them to group themselves into clumps in places of poor illumination. Three or four larger groups of this kind, containing one or two dozen adults with varying numbers of nymphs, were observed at definite sites on the angles between the floor and various walls (two being in corners between two walls and the floor) at distances from one to six feet from the " cage-bed ". Smaller clumps appeared in the angles between walls. The surfaces occupied by these groups of bugs were densely speckled with bug faeces and moderate numbers of eggs. At distances from two to six inches round the sites were observed numerous cast skins.

(d) *Movements of bugs in untreated hut.*

It will be remembered that the original two lots of 20 adult bugs were put into a glass and cardboard shelter on one end of the rabbit's bed. The interior of the shelter was soon speckled with faeces ; the first eggs appeared there, and later the first batch of young nymphs. Nevertheless, the average numbers in the shelter fell to 12 in the 1st and 2nd months and later to 2—5 (see fig. 6). This suggests that bugs, which left the shelter to feed, did not usually find their way back. True, the bugs did not choose the shelter themselves, but were put into it. Therefore, the following experiment was conducted to determine whether bugs are liable to return to the same, freely chosen, resting sites. Adult bugs and old nymphs found in various parts of the hut were marked with spot of paint, without moving them and with as little disturbance as possible. One or two spots of paint of four colours were used in various combinations, to denote the various loci. The positions of the marked bugs were noted and then re-arrangement was determined in two subsequent inspections.

TABLE III.

Movements of marked bugs. The bugs were marked with various colour combinations to indicate their original resting places, A, B, C, . . . etc.

Site	Description	Distance from cage	Bugs marked 4/6/48	Bugs found	
				13/6/48	29/6/48
A	Floor/Wall edge ..	6 in.	4(A)	3(A), 1(C), 1(F)	Nil
B	Ditto	9 in.	3(B)	Nil	Nil
C	Floor/Two-walls corner	6 ft.	5(C)	2(C), 1(F)	1(C)
D	Ditto	1 ft.	2(D)	1(D)	1(A)
E	Ceiling/Two-walls corner	4 ft.	3(E)	Nil	Nil
F	Artificial rest place on cage	0	8(F)	1(F)	Nil
—	Tray of cage ..	0	0	2(B), 1(C), 1(E), 2(F)	2(A), 2(B), 3(C), 1(D), 2(F)
—	Elsewhere	0	0	Nil	1(A), 1(F), 1(F) dead

The observations recorded in Table III indicate a considerable change in position of bugs over periods long enough to embrace one or two feeds. Therefore they show that after feeding the bugs do not necessarily return to their original site but wander until they find a suitable resting place.

Casual observations suggest that they usually walked across the floor of the hut until they reached a wall and then tended to walk along the angle between wall and floor. Sometimes they tended to stop in places of maximum shadow; another tendency was to stop at a corner (*i.e.* when a second wall was reached). Finally, the presence of other bugs (and possibly traces such as faeces or eggs) tended to cause a halt. This would account for the formation of clumps of bugs.

Summary.

An experiment on a semi-practical scale was made to determine the effectiveness of DDT treatment to a new house, in quelling an infestation of bed-bugs introduced with untreated furniture. Two small huts were constructed, one being finally treated with DDT, the other acting as a control. Rabbits introduced nightly represented the hosts and infested rabbit-hutches the furniture.

Forty adult bugs were introduced on the infested hutches in each hut. In the untreated chamber, the bug population soared up to over 400 in six months. In the treated hut the population remained at 10 to 20 up to the end of the experiment (28 weeks); these bugs remained in the untreated hutch. Translating these results to practical conditions, it appears that a bug colony might persist in an untreated bed in a room sprayed with DDT. However, the wandering bugs would be destroyed which would severely check population growth and prevent a heavy and widespread infestation.

Observations in the untreated hut indicated the decline of the first generation adults, the rise and decline of the second generation and emergence of the third.

A few preliminary tests with marked bugs proved that these insects do not usually return to the same site after going to the host to take a meal.

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THE RÔLE OF *AËDES LEUCOCELAENUS* IN THE EPIDEMIOLOGY OF JUNGLE YELLOW FEVER IN BRAZIL.*

By Henry W. Kumm & Nelson L. Cerqueira.

(Plate IX.)

Following the discovery by Soper & others (1933) of the existence of yellow fever without *Aëdes aegypti* (L.), an intensive search was initiated to ascertain what the other vectors of this disease are in nature. In addition to incriminating two species of *Haemagogus*, investigators working in Brazil and Colombia found *Aëdes leucocelaenus* Dyar & Shannon, naturally infected on two occasions. A rhesus monkey, which had been bitten by 16 *A. leucocelaenus* captured at Afonso Arinos in the State of Rio de Janeiro, Brazil, by Shannon, Whitman & Franca (1938), died of typical yellow fever. Six years later Bugher & others (1944) demonstrated the presence of yellow fever virus in a batch of six *A. leucocelaenus* caught in the Chichimene forest near Villavicencio, Colombia.

A number of laboratory transmission experiments have been carried out. Some of them, reported by Waddell (1949), suggest that *A. leucocelaenus* may be an even better laboratory vector of jungle yellow fever virus than *A. aegypti*. Waddell obtained two successful transmissions of yellow fever to rhesus monkeys by the bite of *A. leucocelaenus*. In one, a single mosquito infected at a titer of more than $10^{-7\cdot0}$ transmitted after 22 days of extrinsic incubation, and in another experiment six *A. leucocelaenus* transferred the virus 20 days after their infecting blood meal. Waddell also secured 40 successful transmissions from marmosets to baby mice by the bites of *A. leucocelaenus*, which had incubated virus for periods of from 21 to 52 days.

In certain other studies carried out recently by Kumm & Laemmert (1950) four *A. leucocelaenus* of a lot originally infected on a howler monkey of the species *Alouatta caraya*, when the latter was circulating virus in a titer of $10^{-5\cdot3}$, transmitted yellow fever by bite to a marmoset after an incubation period of 45 days. On the 49th day after infection, five *A. leucocelaenus* of that same lot were still alive. They were killed, ground up and inoculated intracerebrally into groups of six white mice each. Four out of those five insects proved to be infected with yellow fever. In another experiment 10 out of 11 *A. leucocelaenus* originally infected at a titer of $10^{-5\cdot8}$ were shown to contain yellow fever virus by inoculation after an extrinsic incubation period of 28 days.

It is thus evident that *A. leucocelaenus* is a good laboratory vector of yellow fever virus. It has also been found infected in nature on two occasions. However, this species is not abundant in all the areas of Brazil where human cases of jungle yellow fever have been contracted. In extensive captures of day-biting forest mosquitos carried out at Curralinho on Marajó Island near the mouth of the Amazon river by Kumm and Novis (1938), only 13 *A. leucocelaenus* were obtained out of a total of more than 7,000 mosquitos taken with human bait. Similarly in the Ilhéus endemic area of the State of Bahia, *A. leucocelaenus* accounted for less than one tenth of one per cent. of the total catch reported by Laemmert, Castro Ferreira and Taylor (1946). Likewise, in the region around Villavicencio, Colombia, out of a total of 39,107 sylvan mosquitos captured by Bates in the four-year period from 1942 to 1945 inclusive, only 496 *A. leucocelaenus* were secured, which amounted to a scant 1·3 per cent.

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* The studies and observations on which this paper is based were conducted with the support and under the auspices of the National Yellow Fever Service of Brazil and of the Yellow Fever Research Service, which was maintained jointly by the Ministry of Education and Health of Brazil and by the International Health Division of The Rockefeller Foundation.

In the forests of southern Brazil, on the other hand, *A. leucoclaenus* is more common. Table I shows that at Anápolis, Goiaz, it represented 5·8 per cent. of the total catch. Among the forest mosquitos captured at Passos, Minas Gerais, by Causey and dos Santos (1950) it accounted for 4·8 per cent. Near Afonso Arinos and Bemposta in the State of Rio de Janeiro, Shannon, Whitman & Franca (1938) took more *A. leucocelaenus* than *Haemagogus*. If the figures from these five areas in Brazil, where extensive captures of day-biting forest mosquitos have been made, are combined, it will be seen that about half as many *A. leucocelaenus* were captured as *H. spegazzinii* Brèthes.

TABLE I.

Comparative abundance of *Haemagogus spegazzinii* and *Aëdes leucocelaenus* in certain areas of Brazil, where intensive captures of sylvan mosquitos have been carried out.

Areas studied and duration of investigations	Total number of sylvan mosquitos captured	*Haemagogus spegazzinii*		*Aëdes leucocelaenus*	
		Number caught	Per cent. of total catch	Number caught	Per cent. of total catch
Anápolis, Goiaz, December, 1935, to May, 1936 ..	4,315	359	8·3	252	5·8
Curralinho, Pará, November, 1935, to June, 1936	7,379	626	8·5	13	0·2
Afonso Arinos and Bemposta, Rio de Janeiro, January, 1938, to March, 1938	24,304	1,216*	5·0	4,671	19·2
Ilhéus, Bahia, January, 1944, to April, 1945 ..	172,873	9,119	5·3	100	0·1
Passos, Minas Gerais, September, 1945, to December, 1947 ..	73,321	9,788	13·3	3,539	4·8

* Both *H. capricornii* and *H. spegazzinii* are present in the Afonso Arinos and Bemposta areas.

There are other areas in this country, however, where *A. leucocelaenus* is much more abundant. In Rio Grande do Sul, which is the most southerly state of Brazil, many captures of sylvan mosquitos carried out during several of the most favourable months of the year 1949 yielded numerous *A. leucocelaenus* and only a very few *Haemagogus capricornii* Lutz.

Considerable information has also been assembled about the geographical distribution in Brazil of *A. leucocelaenus*. This species has been encountered in 18 of the 20 states, three territories and the Federal District. The apparent absence of *A. leucocelaenus* from the states of Piauí and Rio Grande do Norte has no real significance whatever, because no thorough search has yet been made anywhere in those two states. It is evident, therefore, that *A. leucocelaenus* has a wide distribution throughout this country.

The 124 localities where specimens of *A. leucocelaenus* have been captured are listed below and their position is indicated in fig. 1.

States and Territories.	*Localities.*
Acre	Feijó, Rio Branco, Tarauacá.
Alagoas ..	Atalaia.
Amapá ..	Macapá, Oiapoque.
Amazonas ..	Benjamin Constant, Borba, Fonte Boa, Manáus.
Bahia ..	Belmonte, Carinhanha, Cocos, Ilhéus, Malhada, Paripiranga.

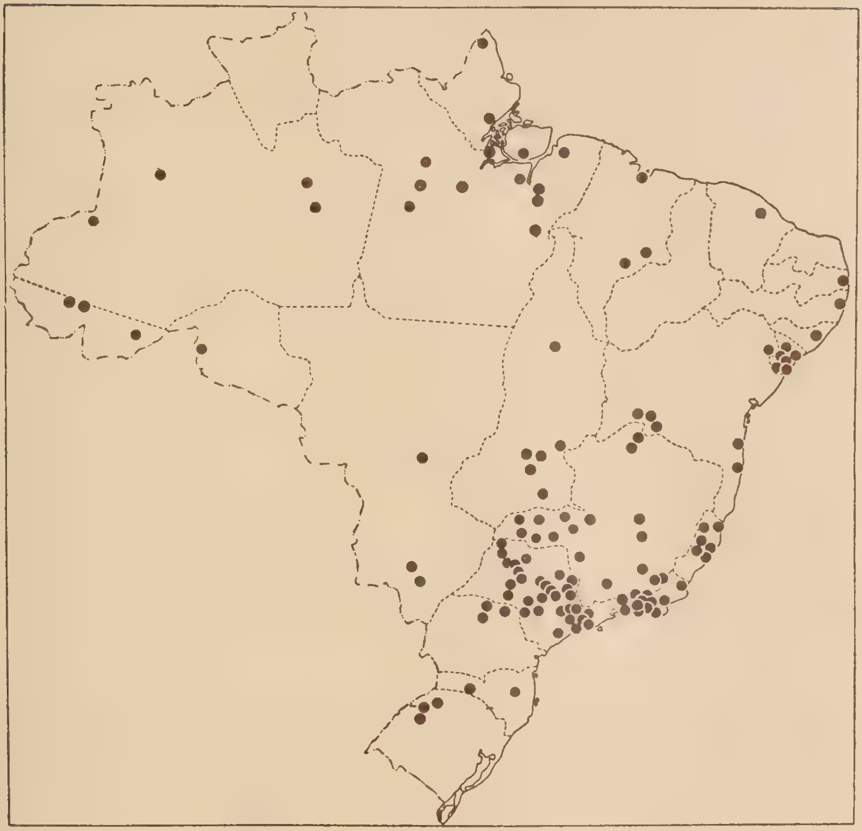

Fig 1.—Localities in which *Aëdes leucocelaenus* has been captured.

Ceará	Maranguape.
Espírito Santo	Santa Leopoldina, Santa Teresa, São Domingos, São Mateus, Vale do Canaã, Vitória.
Federal District	Rio de Janeiro.
Goiaz	Anápolis, Caldas Novas, Corumbá, Formosa, Jaraguá, Porto Nacional.
Guaporé	Guajará Mirim.
Maranhão	Pastos Bons, São João dos Patos, São Luiz.
Mato Grosso	Coronel Ponce, Dourados, Maracajú.
Minas Gerais	Além Paraíba, Barbacena, Cambuquira, Campina Verde, Curvelo, Frutal, Itacarambí, Ituiutaba, Leopoldina, Manga, Monte Carmelo, Passos, Patos, Patrocínio, Pedro Leopoldo, Uberaba, Uberlândia.
Pará	Altamira, Baião, Belterra, Cametá, Curralinho, Gurupá, Itaituba, Marabá, Mocajuba, Santa Isabel, Santarém.
Paraíba	João Pessoa.
Paraná	Cambará, Londrina, Sertanópolis.
Pernambuco	Recife.

Rio de Janeiro	Afonso Arinos, Bem Posta, Cachoeiras, Campos, Caxias, Itaguaçú, Itatiaia, Jurujuba, Magé, Mangaratiba, Nova Iguaçú, Petrópolis, Teresópolis.
Rio Grande do Sul	Santa Rosa, Santo Augusto, São Luiz, Gonzaga.
Santa Catarina	Concórdia, Ibirama.
São Paulo . .	Araraquara, Avaré, Bertioga, Bocaina, Cabreuva, Cerqueira Cesar, Fernandópolis, Indianópolis, Iolanda, Itanhaem, Itapira, Jundiaí, Juquerí, Juquiá, Marília, Matão, Mococa, Mogí das Cruzes, Nhandeara, Nipoã, Parnaíba, Planalto, Pirajú, Ribeirão Preto, São Carlos, Serra da Cantareira, Tambaú, Tanabí, Taquaritinga, Vila Poloni.
Sergipe . .	Arauá, Cotinguiba, Estância, Lagarto, Nossa Senhora das Dores, Simão Dias.

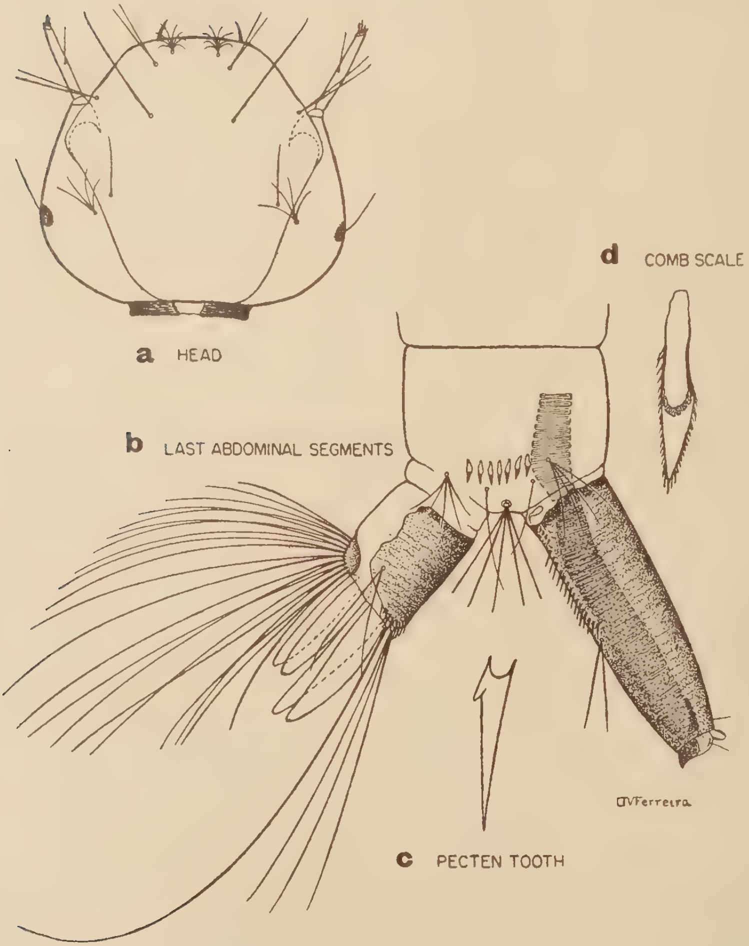

Fig. 2.—Larva of *A. leucocelaenus*.

Until it was incriminated as a natural vector of jungle yellow fever, *A. leucocelaenus* was not of any particular interest to medical entomologists. Consequently, no good diagram is available in the literature illustrating the characteristic morphology of the adult. For that reason a coloured plate (Pl. IX) is included, which brings out the bluish black coloration of the thorax, abdomen and legs of this mosquito, the narrow median longitudinal stripe of silvery white scales on its mesonotum and the spot of white scales on the outer third of the femora of each middle leg. A diagram of the larva, which was described but not drawn by Komp (1938), has also been prepared.

Summary.

Aëdes leucocelaenus transmits yellow fever virus readily in the laboratory and has twice been found infected in nature.

Except in south Brazil this species is much less common than *Haemagogus spegazzinii* among day-biting sylvan mosquitos captured with human bait.

A. leucocelaenus has a wide distribution throughout the forested area of this country.

As a natural vector of jungle yellow fever in the northern part of Brazil it is of secondary importance when compared to *H. spegazzinii.*

Acknowledgements.

It is a pleasure to acknowledge the valuable help received from John Lane, Lério Gomes and Arlindo Pinto de Souza in gathering information about the geographical distribution of *A. leucocelaenus* in Brazil. We are also indebted to Dr. Marston Bates and G. B. Fairchild for furnishing unpublished data about the comparative prevalence of various species of sylvan mosquitos captured in the Villavicencio area of Colombia and close to Anápolis, Goiaz, Brazil.

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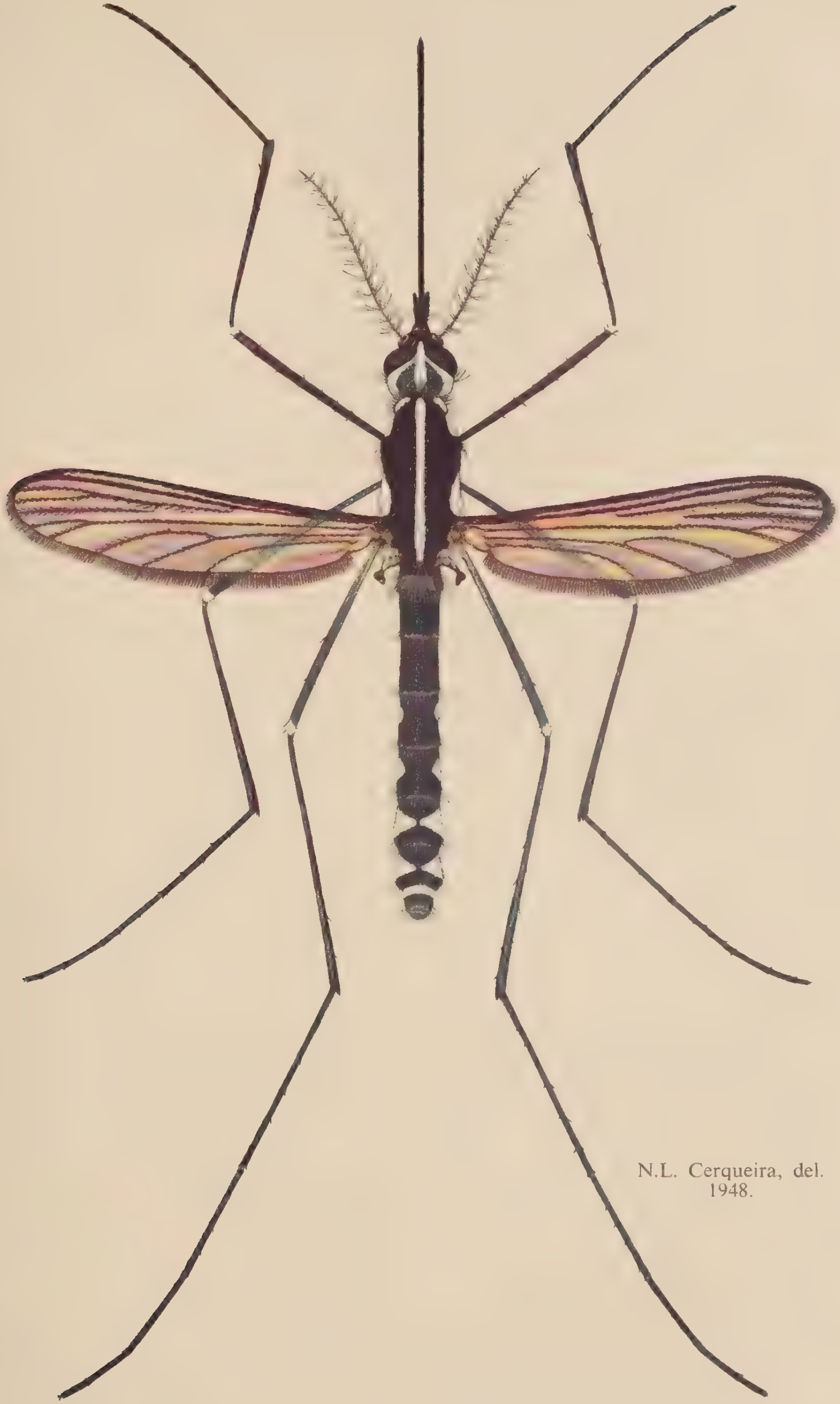

N.L. Cerqueira, del.
1948.

Aëdes leucocelaenus Dyar & Shannon.

FIELD OBSERVATIONS ON THE ACTIVITY OF *PHEIDOLE*.

By P. Belinda Kemp, B.A.

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The object of these investigations was to observe the foraging activities of ants of the genus *Pheidole*. It has been shown by J. Ford (Half-yearly Rep. E. Afr. Tsetse Res. Org., 1939) that the larger species of this genus are among the principal predators of tsetse pupae. W. H. Potts (unpublished report, E. Afr. Tsetse Res. Org., 1938) exposed tsetse pupae in various sites and showed higher losses from unburnt bush than from bush which had been burnt annually, presumably through the action of predators. Further work, however (J. Ford and E. F. Whiteside, unpublished report, E. Afr. Tsetse Res. Org., 1947), has shown that these results are only repeated at certain times of year. It was therefore decided to investigate the foraging activities of *Pheidole* in different types of country and to observe any possible alteration in the food supply of this ant under conditions of fire exclusion.

Soil and Vegetation.

The work was carried out in country which has been reserved for research work by the E. African Tsetse Research Organisation at Shinyanga. This district is in the Central Province of Tanganyika, at an altitude of 3,800 feet. The area consists of very gentle slopes which drain into a system of seasonally swampy depressions. Granite underlies much of the area. The soils conform to the catena of Ukiriguru which has been described by Milne (1947). The ridges are comprised of red sandy loams which grade down to a ferruginous gravel at depths varying from 1–4 feet. On lower slopes there is a grey soil of highly mineralised granitic sand overlying an excessively hard pan which occurs at a depth of 3 to 18 inches. A heavy black clay which cracks in the dry season is found in the seasonally swampy depressions.

The vegetation has been described by Burtt (1942). Glover (unpublished report, E. Afr. Tsetse Res. Org., 1950) has studied the area in more detail and the sites used for his vegetation analyses were selected for the work on *Pheidole*. This work was carried out in three types of country : (i) on deep red soil in an area which is burnt annually and partly grazed by cattle ; (ii) on similar red soil about two miles to the east, in an area which has been protected from fire and grazing for a period of 15 years ; (iii) on grey soil in an area protected from fire and grazing for 11 years. All sites are grazed to some extent by game animals. The thicket on red soils was made up of *Commiphora fischeri*, *C. schimperi* and *Combretum zeyheri*, with other species. On grey soil, *Abrus schimperi* was the most abundant shrub. Many species of trees and shrubs, including *Markhamia obtusifolia*, *Dichrostachys glomerata* and *Grewia* spp., were common on all types of country. Glover has shown that the effect of excluding fire is to increase the density of the thicket ; there was a greater number of shrubs 3–6 ft. in height in the protected area. The grass cover in the protected country was half that of the area burnt annually and subjected to moderate grazing.

In this district there are two rainy seaons in the year, December to January and March to April. The maximum temperature for the year is reached at the end of the dry season in October, when the monthly average is about 33°C. In July, at the end of the long rains the average monthly temperature is at a minimum. Some more detailed measurements were made on the sites, and these show that apart from a higher average temperature during the dry season, the temperature at this time of year rises more rapidly in the morning reaching a maximum earlier in the day. Records on the sites in October showed a maximum temperature 3° higher and two hours earlier than in similar situations in July. When the area is burnt annually the thicket tends to be more compact and the intervening ground devoid of leaf litter; temperatures recorded in thicket and open ground therefore show greater contrast on the red soil sites burnt annually than on red soil in the area protected from fire. The more open thicket on the unburnt red soil sites does not provide good shelter and temperatures are higher here than on red soil sites burnt annually or in the dense thicket found on grey soils.

Activities of Foraging Ants.

Some preliminary investigations showed that methods devised to count individual ants foraging on the surface in a given area did not provide adequate data. A very much larger sample was obtained by attracting ants to a bait and the following conclusions are based on this method.

The baits consisted of fresh raw lean meat, which was cut into cubes of approximately 3 gm. These baits were placed at the base of flagged wire markers where a small area was cleared of grass and leaves; 25 baits were laid on each plot and there were two plots in each type of country selected. The baits were laid in 5 rows of 5 and all 2½ metres apart, baits therefore covered an area of 10 × 10 metres. The baits were left in position for ¾ hour, after this time they were picked up and dropped into a jar of 1 per cent. formalin as rapidly as possible. The baits from each plot were collected in a single container. The ants collected by this method were those running on the meat or adhering to it with their mandibles, the large number of ants congregated in the vicinity would not be collected. The ants were counted when all the field work had been completed. The baiting of each plot took place over a period of two days, at intervals of six hours between each baiting to ensure that ants aggregated in the vicinity had dispersed. The baits were laid at 6, 12 and 18 hours on the first day and 9, 15 and 21 hours on the second day. These counts were made at the end of the rains in July, and repeated in October at the end of the dry season.

Before discussing the results, it is necessary to consider the method by which they were obtained in more detail. The graphs are drawn from figures of the total ants collected from 50 baits, which had been laid down on two plots of 25 baits in each type of country. The numbers of ants found on a single bait varied greatly. There is no doubt that if food is found by a foraging ant the other members of the nest are "informed" and attracted to it; this is easily observed. Within a limit, therefore, the number of ants on a single bait may depend on the time at which that bait was first discovered. For example, in a plot of 25 baits 456 *Pheidole crassinoda* Emery, were collected and these were distributed as follows: 0, 31, 0, 2, 1, 0, 0, 3, 1, 242, 0, 0, 67, 0, 4, 0, 0, 42, 33, 0, 6, 6, 18, 0, 0. This is a typical example and the same is true for other species of ants. A similar result is obtained if the baits are exposed for a greater length of time. Another experiment was carried out to discover the relation between the baits and the nest. Forty baits were arranged in eight lines radiating out from one entrance to a nest of *Pheidole*. This operation was carried out at 8.00 a.m. when a great many ants were active. There were three definite routes from the nest being followed by the ants at the time, but baits in the line of these paths were not attacked more readily than those elsewhere. Although ants were working on heaps of debris which had been discarded from the nest, baits in this position were ignored. The number of ants found on a bait must depend on the complex behaviour pattern of the worker ant.

The majority of ants attracted to these baits were members of the sub-family MYRMECINAE (Arnold, 1915). These results are principally concerned with the two larger species of *Pheidole*, *P. sculpturata* Mayr and *P. crassinoda* Emery. These species have been classed together as it is not possible to distinguish the worker ants, and other castes were rarely attracted to the baits. The ants most commonly found on the baits are given in Table I which shows their relative abundance.

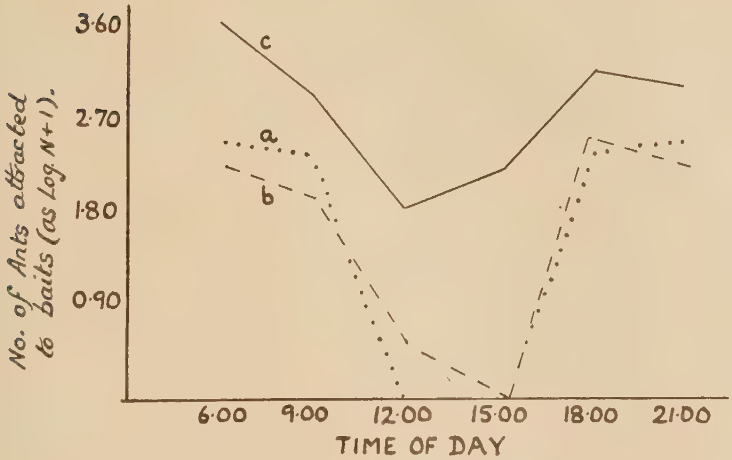

Fig. 1.—*Pheidole sculpturata* and *P. crassinoda*. The activity of foraging ants in July, at the end of the rains. (*a*) on red soil burnt annually; (*b*) on red soil, unburnt; (*c*) on grey soil, unburnt.

TABLE I.

Total ants collected by baiting.

Species	July	October
Pheidole sculpturata with *P. crassinoda*	10,427	3,405
Pheidole sp. A	90	166
Pheidole sp. Q	643	846
Tetramorium sp. F	155	30
Monomorium salomonis var. *diserta*	678	1,463
Monomorium sp. G	513	1,332
Ocymyrmex weitzeckeri	76	427

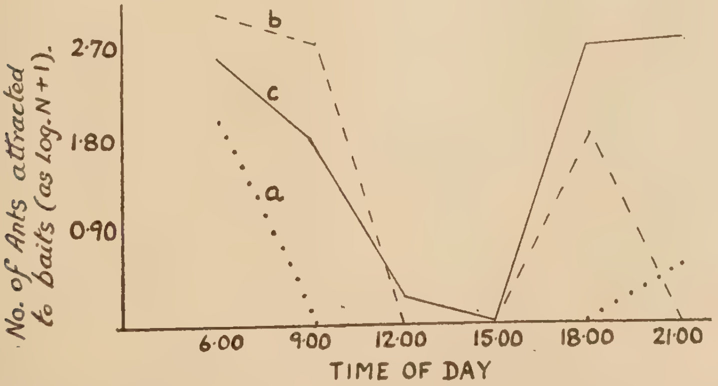

Fig. 2.—*Pheidole sculpturata* and *P. crassinoda*. The activity of foraging ants in October, at the end of the dry season. (*a*) on red soil, burnt annually; (*b*) on red soil, unburnt; (*c*) on grey soil, unburnt.

The numbers of *Pheidole sculpturata* and *P. crassinoda* attracted to the baits at different times of day are illustrated by the graphs in figs. 1 and 2. These results were recorded as the number of ants collected from a total of 50 baits laid on each site for every baiting period. In all types of country, the greatest numbers of *Pheidole* were recorded in the morning and evening. In both July and October, *Pheidole sculpturata* and *P. crassinoda* were most active at dawn and for a few hours after sunset. There were much fewer ants foraging at midday, and at the end of the dry season activity ceased almost completely between noon and 4.00 p.m. Although baiting was not carried out over a period of 24 hours, observations in the field have been made at all times. There appeared to be no ants active just before dawn. At 15 minutes before sunrise the first ants are seen leaving the nest, moving slowly and in increasing numbers ; by sunrise they are already scattered over a wide area. After sunset their activity extends over a long period, probably until a few hours before dawn. Twenty-five baits, which had been laid at midnight on the red soil sites, attracted a total of 37 *Pheidole* workers. The activity of these ants was also observed at 2.00 a.m. when they were still abundant on the surface of the ground. Conditions in the bush must be most variable at this time, depending on winds, local air currents and the temperatures reached by the surrounding vegetation and ground during the day ; it would be necessary to make a great number of observations before any conclusions could be reached concerning the time and conditions under which *Pheidole* ceased to be active on the surface. The time at which activity commences in the mornings is very much more regular.

During July there was no difference between the numbers of ants collected from the red soil sites in burnt and unburnt bush, but at this time of year there was a noticeably larger number of ants attracted to the baits on the grey soil sites at all times of day. In October, the numbers of *Pheidole* found on the red soil and grey soil sites in the area protected from fire were more nearly equal, but there were fewer ants found on the red soil sites in country which was burnt annually.

The other species of this genus, *Pheidole* sp. A and *Pheidole* sp. Q occurred more locally, but resembled *P. sculpturata* and *P. crassinoda* in being active mainly in the morning and evening.

In contrast to *Pheidole*, ants of the genus *Tetramorium* showed a peak in their period of activity about three hours after sunrise and three hours before sunset (fig. 3). In July, during the cooler part of the year, they were found foraging throughout the day, but at the end of the dry season there was a cessation of activity at midday. Ants of the genus *Monomorium* resembled *Tetramorium* in this respect.

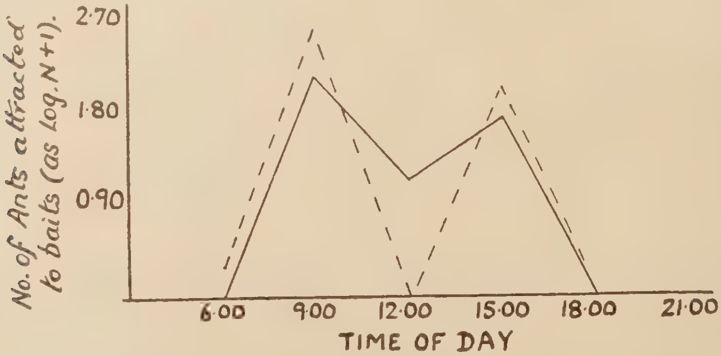

Fig. 3.—*Tetramorium* sp. The activity of foraging ants. Continuous line in July ; broken line in October.

It is of interest to compare these species with *Ocymyrmex weitzeckeri* Emery. This ant was attracted to the baits in greatest numbers during the middle of the day, especially in October at the end of the dry season, at a time when other ants were rarely seen (fig. 4). *Ocymyrmex* is conspicuous for the great speed with which the workers throw out sand when constructing the nest. It is common on sandy ground frequently in open spaces along roadside and pathways, where workers are seen excavating at lightning speed during the hottest periods of the day.

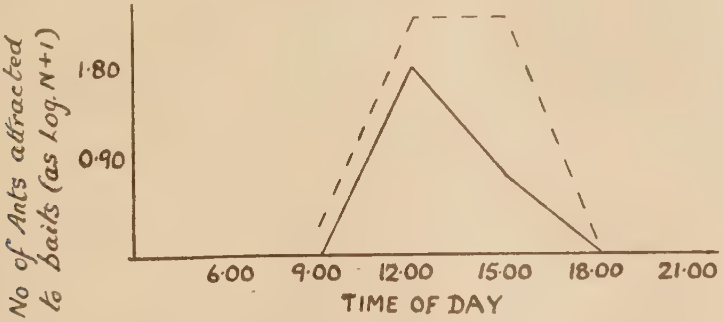

Fig. 4.—*Ocymyrmex weitzeckeri.* The activity of foraging ants. Continuous line in July; broken line in October.

Food Supply of *Pheidole.*

Grass seeds form the principal food of *Pheidole sculpturata* and *P. crassinoda* and, although these ants are omnivorous, insect material probably forms a very small proportion of their normal diet. A number of the piles of refuse cast out from the nests of *Pheidole* were examined and were found to be principally made up of the seed husks from grasses such as *Brachiaria*, *Rhynchelytrum* and *Tragus berteronianus.* There was only a small amount of insect debris in these, which included heads of termites (*Termes* and *Trinervitermes*). Collections of foraging ants were also made, and they were most frequently found to be carrying grass seeds or termites. It can be concluded that grass seeds are the most important food of the ant *Pheidole.* When seeds have been collected by the ants, they are taken into the nest and stored in chambers underground.

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In making vegetation analyses of this area, Glover included estimations of the percentage grass cover on the ground. These results are given in Table II. Estimations of seed production are in close agreement with records of the basal grass cover; in a series of samples the weight of seed collected from red soil, unburnt country (56 gm.) was half that collected from a similar area on red soil burnt annually (113 gm.). Records of the basal grass cover therefore give an indication of the plant food available for *Pheidole*; the amount of grass found in unburnt country being half that found on the sites in country burnt annually. This suggests that there is a reduction in the food available for *Pheidole* under conditions of fire exclusion.

TABLE II.

The percentage of basal grass cover.

	October, 1948	March, 1949	October, 1949
On red soil site, burnt annually	2·696	3·676	2·006
On red soil site, unburnt	1·172	1·193	0·628
On grey soil site, unburnt	0·905	1·217	0·823

Conclusions.

The counts which have been described give an estimate of the number of ants foraging on the surface of the ground, and their periods of activity during the day. No indication is given of the size of the population of ants in different types of country, since it is not known what percentage of the inmates of the nest are concerned with foraging. An estimation of the number of nests was not possible as these are extensive underground structures with many openings. For *Pheidole*, it has been shown that there is less plant food available in the areas which have been protected from fire, and under these conditions the greatest numbers of ants were attracted to the baits. The question whether this represents a larger ant population or an increase in the intensity of the search for food remains unanswered. The significant fact so far as tsetse pupae are concerned is that the most intensive foraging by ants is to be found under these conditions.

Summary.

A method is described for observing the numbers of ants foraging on the surface of of the ground by attracting them to baits of raw meat. The general area is described briefly and sites were selected for the work in three types of country.

A total of 50 baits were laid on each site in July and October. *Pheidole sculpturata* and *P. crassinoda* were the most abundant ants found on the baits. These were active mainly in the morning and evening, fewer ants were found at midday during the dry season. Comparing the numbers collected on the baits, more *Pheidole* were found in July than in October, and in July more ants were found on the unburnt grey soil sites than elsewhere. On the red soil sites there was a difference between burnt and unburnt country only in October, when more ants were found on the red soil in unburnt country. *Pheidole* is compared with three other genera of ants commonly found on the baits.

The food of *Pheidole* is mainly grass seeds ; some reference is made to the supply of grass seeds in different types of country and the relation between this and the numbers of foraging ants.

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THE MOSQUITOES OF BWAMBA COUNTY, UGANDA.*

VIII.—RECORDS OF OCCURRENCE, BEHAVIOUR AND HABITAT.

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At the time of writing, 1949, field work on mosquitoes has been carried on in Bwamba County, western Uganda, for a period of ten years, with very few intermissions. The topography and vegetation of this area have been described elsewhere (Haddow, 1945a). The main effort has centred round the search for forest vectors of yellow fever, but much work has also been done in native plantations, huts, etc. While genera such as *Aëdes* and *Taeniorhynchus* have, inevitably, required special attention, records of all mosquitoes encountered have been kept throughout the investigation, whether or not they seemed likely to be involved in the local epidemiology of yellow fever. Certain sections of the work have already been published (Mahaffy & others, 1942 ; Haddow, 1945a, 1945b, 1945c, 1946 and 1948 ; Haddow & others, 1947 ; Haddow & Dick, 1948 ; Haddow & Mahaffy, 1949), but a great part of it has not as yet been submitted.

The number of species encountered in Bwamba has been quite exceptional, the present list standing at 160 forms, in addition to which we know of at least ten others which we are not yet able to describe formally. The richness of the fauna may be gauged from the fact that the total East African list comprises, at the moment, only 296 forms, of which (at least in so far as East Africa is concerned) 31 are confined to Bwamba. By " East Africa " we mean Kenya, Uganda, Tanganyika and Zanzibar. The Bwamba sample is large, the numbers of specimens examined standing at present at about 50,000 larvae and 300,000 adults. In view of the fact that existing literature contains but little information concerning the habits and distribution of the great majority of these mosquitoes, it has seemed worth-while to attempt to set forth, in the briefest form compatible with clearness, what is known of their behaviour and natural habitats in this area.

Many new forms have been encountered in Bwamba County, and these have been described in separate communications (van Someren, 1948, 1949, 1950a, b, c, 1951 ; Haddow & van Someren, 1950 ; Haddow, 1946). Quite apart however from the study of new species, much information has been gained about hitherto unknown stages, and more particularly about the important subject of variation within species (van Someren, 1946a, b, 1947, 1948, 1949, 1950b & 1951 ; Haddow, 1946). In this connection it may be mentioned that, in almost every case where a long series of specimens has been available for study, it has been found that the range of variation occurring within a species is considerable. This has frequently led to great difficulty in determination, and in some cases provisional names have had to be allocated (sometimes over a period of years) before final identification was possible. In certain instances, which are noted individually below, the writers are still in doubt. In others, the study of long series has led to dubiety about the validity of forms formerly regarded as distinct, or at least to dubiety concerning the validity of their alleged diagnostic characters. Such cases occur, for example, among the *Taeniorhynchus* (*Coquillettidia*) spp. of the *aurites-aureus-microannulatus* group, where the various forms may represent some kind of polymorphism rather than a series of distinct species, and among the *Eretmapodites chrysogaster* group where, though it seems likely that the various species and subspecies are valid, the male terminalia form the only reliable diagnostic character. A section on variation has, therefore, been included in the case of each mosquito which has shown interesting features in this respect.

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In the course of work in Bwamba the writers have (as mentioned above) encountered at least ten forms which were almost certainly new, but of which the material has been too scanty to permit safe designation of new species or subspecies, or in which the only specimen has been lost, or so damaged that description was impossible. For the benefit of future workers in western Uganda it has seemed worth-while to include a brief note, under each genus, indicating that such forms are present and to be sought for.

In the interest of brevity it has been found necessary to use certain contractions in the succeeding notes. " Forest ", where not otherwise qualified, means the forest undergrowth and " Canopy " the forest canopy. " Plantations " means banana plantations which, however, as noted elsewhere (Haddow, 1948) may be of a very mixed nature, including various other cultivated plants. The heading " Females, not biting " has been used as some of the specimens in this category were taken while resting, others while in flight. The group, " Females, biting " includes all species taken in catches in which a human " bait " was offered, and in which collection was confined to specimens alighting on the bait. It is possible that some of the rarer species here included (and more particularly the genera *Uranotaenia* and *Ficalbia*) merely alighted on the skin without actually biting (Haddow & others, 1947).

It has been necessary to use terms descriptive of prevalence, so that the relative abundance of a species in its various habitats may be gauged. These terms, arranged in order of magnitude, are as follows : " abundant ", " very common ", " common ", " not uncommon ", " uncommon ", " scarce ", " rare ", " very rare ". Two further terms are used in a slightly different manner, " occasional " indicating an irregularity of occurrence in the catches, and " exceptional " indicating behaviour lying quite outside our normal experience of the species concerned.

Bwamba presents a remarkably wide variety of different environments, ranging from dense lowland rain-forest through cultivated country to open grassy uplands, mountain forest, mountain bamboo and alpine flora (Haddow, 1945a). With regard to mosquito distribution, two main divisions may be recognised. The lowland area includes the rain-forest, the adjoining cultivated areas, and the foothills of the Ruwenzori Mountains up to a height of about 5,500 feet. The highland area comprises the grassland, cultivation and mountain forest above this level. As the mosquito faunas of these two main divisions are strikingly different, only a few species being common to both, a note about this has been included with each mosquito discussed. In addition, published records of mosquito distribution in East Africa being scanty, a note on this subject has been added in cases of interest.

The nomenclature and systematic arrangement used by Edwards (1941) has been followed, notes being given in the few cases where the writers do not agree with his conclusions. The individual species may now be discussed.

1. **Anopheles (Anopheles) coustani** Lav. (*S. str.*)

Lowlands only.

FEMALES. *Biting by night*—forest and open ground, scarce.

VARIATION. One female with rather paler legs than usual, the ochreous line below on the femora, tibiae and first tarsal segment wider, extending on to anterior surface, and posterior surface nearly all ochreous.

NOTE. In view of the fact that all varieties of *A. coustani* occur in Bwamba, and that sometimes two or more have occurred in a single catch, it is felt that the taxonomy of this group deserves further attention.

2. **A. (A.) coustani** Lav., West African form (Evans, 1938, key, p. 76).

Not known in East Africa outside Bwamba lowlands.

FEMALES. *Biting by night*—forest and plantations, scarce.

3. **A. (A.) coustani** var. **ziemanni** Grünb.

Lowlands only.

FEMALES. *Biting by night*—forest, plantations, huts and tents, not uncommon ; canopy, rare. *Not biting*—huts and tents, scarce.

VARIATION. The two apical white bands on palps sometimes very broad and occasionally not separated by a band of dark scales. Wing markings vary slightly, sometimes very dark ; pale spot on costa and apical pale spot very small.

4. **A. (A.) coustani** var. **tenebrosus** Dönitz.

Within Uganda recorded from Bwamba lowlands only.

FEMALES. *Biting by night*—forest, very rare (only 1 taken).

5. **A. (A.) paludis** Theo.

Lowlands only. Not known in East Africa outside Uganda.

FEMALES. *Biting by day*—forest and plantations, occasional ; canopy, scarce. *By night*—forest and plantations, not uncommon ; canopy and open ground, occasional. *Not biting*—plantations, occasional.

6. **A. (A.) obscurus** (Grünb.).

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, rare. *By night*—forest, rare.

MALES. Forest, rare.

VARIATION. A male and female examined are as the Uganda variety described by Evans (1938) with fifth hind tarsal segment all white, fourth white at base, and male terminalia as in the Belgian Congo form.

7. **A. (A.) implexus** (Theo.).

Both lowlands and highlands.

FEMALES. *Biting by day*—forest and plantations, occasional; canopy, scarce. *By night*—forest, not uncommon ; plantations, occasional ; canopy and open ground, scarce. *Not biting*—forest and plantations, occasional.

VARIATION. Tarsal markings very variable, as noted by De Meillon (1947). None with white basal band on first hind tarsal segment, recorded (but not figured) by Evans (1938). Some have femora very pale with narrow dark bands, the largest just before tip.

8. **A. (Myzomyia) nili** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, scarce. *By night*—plantations and huts, scarce.

9. **A. (M.) funestus** Giles.

Lowlands only.

Females. *Biting by day*—forest and plantations, uncommon ; canopy, open ground, huts and tents, scarce. *By night*—huts and tents, common ; forest and plantations, not uncommon ; canopy and open ground, uncommon. *Not biting*—huts and tents, common ; forest, uncommon.

Males. Forest and huts, uncommon.

10. **A. (M.) marshalli** (Theo.).

Lowlands only.

Females. *Biting by night*—forest, rare.

Variation. Tibiae very indistinctly pale at apices.

11. **A. (M.) moucheti** Ev.

Lowlands only. Not known in East Africa outside Uganda.

Females. *Biting by day*—forest, scarce. *By night*—forest, scarce ; canopy, rare.

12. **A. (M.) hargreavesi** Ev.

Not known in East Africa outside Bwamba lowlands.

Females. *Biting by day*—forest, occasional. *By night*—forest, not uncommon ; canopy, plantations and open ground, occasional.

Variation. Sometimes dark ring separating the two apical white bands on palps very narrow (occasionally practically absent).

13. **A. (M.) demeilloni** Ev.

Lowlands only.

Females. *Biting by night*—forest and plantations, scarce ; canopy, rare.

14. **A. (M.) keniensis** Ev.

Lowlands only.

Females. *Biting by day*—forest, occasional. *By night*—forest and plantations, occasional ; canopy, rare.

15. **A. (M.) gambiae** Giles.

Lowlands only.

Females. *Biting by day*—forest, huts and tents, not uncommon ; canopy, plantations and open ground, scarce. *By night*—forest, huts and tents, abundant ; canopy, very common ; plantations and open ground, common. *Not biting*—forest, huts and tents, abundant ; plantations, common.

Males. Forest, very common ; huts and tents, common.

Larvae and Pupae. Ground pools in forest, plantations and open ground, very common ; slow streams, rare.

Variation. Showing the usual variation in size and colour. Leg markings very variable. Apical white band on palps unusually broad—half and sometimes three-quarters of fourth segment white. One specimen with an abnormally pale wing, dark areas being reduced to small spots ; costa and first vein nearly all pale but with three short dark areas in middle and a small one at tip.

Note. In forests, the distribution of *A. gambiae* is very uneven ; it is usually abundant in uninhabited areas, but may be scarce or apparently absent in localities where huts are common. In such circumstances, it is apparently deflected from the forest to its more usual hut habitat. With regard to the record of larvae from forest ground pools, it should be noted that, apart from their situation in forest (often miles from the nearest dwelling), these were typical sunlit puddles. Larvae have not been found in shaded forest pools. The record from slow streams is of considerable interest. Larvae in this case were found in tepid water of high salinity flowing from hot springs.

16. **A. (M.) pharoensis** Theo.

Lowlands only.

FEMALES. *Biting by day*—forest, occasional. *By night*—forest, canopy, plantations, huts and tents, occasional. *Not biting*—huts and tents, occasional.

VARIATION. Legs paler than usual—white, with well-defined spots or patches of dark scales. First segment of all tarsi white with a distinct (or indistinct) patch of dark scales below, just proximal to apical white band.

NOTE. In Bwamba the occurrence of this species is rather peculiar. It may occur in quite large numbers for a night or two, after which it disappears—sometimes for a prolonged period.

17. **A. (M.) squamosus** Theo.

Lowlands only.

FEMALES. *Biting by night*—plantations and open ground, rare (only 2 taken).

18. ***Megarhinus brevipalpis** subsp. **conradti** Grünb.

Lowlands only.

FEMALES. *Not biting*—forest, not uncommon ; house, occasional.

MALES. Reared material only.

LARVAE AND PUPAE. Tree-holes in forest, not uncommon ; tree-holes in open ground and receptacles in plantations, occasional.

VARIATION. Supra-alar and scutellar bristles usually black but those on scutellum occasionally bright golden. Abdominal tergites IV–VII usually dullish purple but sometimes brilliant metallic green, shading to blue towards apex of abdomen. White patch on abdominal sternite V of males occasionally interrupted in middle.

NOTE. The house record is of interest. The house concerned is on the edge of a belt of forest, and about six females have been taken in it over a period of three years.

19. **M. barbipes** Edw.

Lowlands only. Apart from Toro District, Uganda, recorded only from Kaimosi and Kakamega, Kenya.

FEMALES. *Not biting*—forest, occasional ; house, exceptional.

VARIATION. For variation (and description) of female and early stages, see van Someren (1946b).

NOTE. One female has been taken in the same house in which *M. brevipalpis* subsp. *conradti* occurred.

20. **M. ruwenzori** van S.

Known only from Bwamba highlands and Kigezi highlands (south-west Uganda).

MALES AND FEMALES. Reared material only.

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*For remarks on the status of this generic name see Muspratt, p. 355.

LARVAE AND PUPAE. Bored bamboos, mountain forest, not uncommon.

VARIATION. One female from Ruwenzori and one from Kigezi differ from type as follows : Patch of blue scales on sternopleuron and lower mesepimeron absent, only white scales being seen, though in certain lights they appear light brown as in type. The Ruwenzori specimen differs also by having third fore-tarsal segment white all round on basal half, and segments 4 and 5 of fore tarsi whitish below for full length. The Kigezi specimen had darker legs, first tarsal segment of all legs dark all round, or with a faint white patch below at base, and white markings on second fore-tarsal segment reduced.

NOTE. Although these specimens lack bright blue scales on the pleuron, the general colour and ornamentation conforms to the *M. ruwenzori* pattern and agrees with this species in differing from *M. lutescens* Theo. and *M. nairobiensis* van S. by having white scales on *ppn*. The scales on *ppn* of *M. ruwenzori* may have a yellow sheen, but they are never the bright golden colour of those found in the other two species.

NOTE ON A PROBABLY UNDESCRIBED SPECIES. A female *Megarhinus*, almost certainly a new species, was reared from a pupa taken in a tree-hole in the lowland forest. The specimen was unfortunately smashed and no other has been obtained. This very striking mosquito obviously belonged to the same group as *M. nairobiensis* van S.

21. **Harpagomyia taeniarostris** Theo.

Both lowlands and highlands.

FEMALES. *Not biting*—plantations, common ; forest scarce ; canopy and huts, very rare.

MALES. Reared material only.

LARVAE AND PUPAE. Plant-axils in plantations, very common ; plant-axils in forest, occasional.

22. **H. fraseri** Edw.

Not known in East Africa outside Uganda.

FEMALES. *Not biting*—forest, very rare.

23. **Uranotaenia alboabdominalis** Theo.

Lowlands only.

FEMALES. *Biting by day*—forest, rare. *Not biting*—forest, not uncommon.

MALES. Forest, rare.

VARIATION. Some specimens quite typical except that femora and tibiae have small but distinct creamy patches at apices. One female differs by having no basal white markings on tergites VI and VII.

NOTE. This is the only species of *Uranotaenia* which the writers have taken actually engorging on human blood.

24. **U. bilineata** var. **fraseri** Edw.

Lowlands only.

FEMALES. *Not biting*—forest, occasional.

NOTE. In view of the fact that three varieties of *U. bilineata* occur in Bwamba, it seems that it might be preferable to regard it simply as a variable species rather than to subdivide it into a series of named varieties.

25. **U. bilineata** var. **connali** Edw.

Lowlands only.

FEMALES. *Not biting*—forest, occasional.

VARIATION. Leg markings typical but abdominal markings like those of var. *obsoleta* Edw. (1941) ; only tergites IV–VII with creamy basal markings which (although well separated from the sides) are in the form of bands rather than spots.

26. **U. bilineata** var. **obsoleta** Edw.

Lowlands only.

FEMALES. *Not biting*—forest, occasional ; canopy, very rare.

MALES. Forest, scarce.

27. **U. balfouri** Theo.

Lowlands only.

FEMALES. *Not biting*—forest, occasional ; canopy, rare.

MALES. Forest, rare.

28. **U. candidipes** Edw.

Not known in East Africa outside Bwamba lowlands.

FEMALES. *Not biting*—forest, rare (only two taken).

VARIATION. Head scales all yellow. Third hind-tarsal segment white on distal third only ; fore and mid tarsi with segments 4 and 5 and tip of 3 ochreous. One specimen with dark basal bands on sternites VI and VII.

29. **U. ornata** var. **musarum** Edw.

Both lowlands and highlands.

FEMALES. *Not biting*—plantations, common ; forest, not uncommon.

MALES. Forest and plantations, occasional.

LARVAE AND PUPAE. Plant-axils in plantations, very common ; plant-axils in forest, occasional ; tree holes in forest, exceptional.

VARIATION. Head often with a large patch of black scales in middle, and sometimes vertex completely black (leaving only very small patches of yellow scales at sides). Abdominal tergites III and IV dark, but IV sometimes with a narrow yellow basal band ; sternites often largely dark.

30. **U. yovani** van S.

Known from Bwamba lowlands only.

MALES AND FEMALES. Reared material only.

LARVAE AND PUPAE. Plant-axils (*Pandanus*) in forest, not uncommon.

VARIATION. Larvae and pupae distinct, but adult indistinguishable from *U. ornata* var. *musarum* ; head usually darker and abdominal markings bolder, but variable.

31. **U. nigripes** (Theo.).

Not known in East Africa outside Bwamba lowlands.

FEMALES. *Not biting*—forest, very rare.

32. **U. mashonaensis** Theo.

Lowlands only.

FEMALES. *Not biting*—forest, occasional ; canopy and plantations, rare.

MALES. Forest, scarce.

33. **U. nigromaculata** Edw.

Lowlands only. Not known in East Africa outside Uganda.

MALES. Forest, very rare.

34. **U. fusca** Theo.

Lowlands only.

FEMALES. *Not biting*—forest and plantations, scarce.

35. **U. garnhami** van S.

Known only from Bwamba highlands and Kigezi highlands (south-west Uganda).

LARVAE. Bored bamboos in mountain forest.

36. **U. shillitonis** Edw.

Both highlands and lowlands. Not known in East Africa outside Uganda.

MALES AND FEMALES. All reared material, with the exception of a single male from lowland forest.

LARVAE AND PUPAE. Bored bamboos in mountain forest, very common.

NOTE. The lowland record is quite exceptional, the real home of this mosquito being in the higher mountain forest.

37. **Aëdomyia africana** Neveu-Lemaire.

Lowlands only.

FEMALES. *Biting by night*—canopy, very rare (only one taken).

VARIATION. With yellow instead of white scales in middle of head, and scales on first flagellar segment of antenna white.

38. **Theobaldia (Allotheobaldia) longiareolata** (Macq.).

Not known in East Africa outside Bwamba lowlands.

FEMALES. *Not biting*—tent, open country, very rare (only one taken).

39. **T. (Theomyia) fraseri** (Edw.).

Lowlands only.

FEMALES. *Biting by day*—forest, occasional.

MALES. Forest, scarce.

LARVAE AND PUPAE. Tree-holes in forest, not uncommon.

VARIATION. Proboscis occasionally wholly dark, but usually distal two-fifths golden. Scutum often with a band of pale yellow scales round front margin, meeting lateral pale lines at scutal angle. Pale lines on scutum broader in male than in female. Fore and mid tarsi often with a broad creamy line below on segments 1 and 2, sometimes extending to upper surface on basal third to form an indistinct band on first fore-tarsal segment.

NOTE. The interior of the larval thorax is bright blue in life. The larvae have a characteristic habit of swimming in small circles, well below the surface, using the mouth-brushes only, and with the long siphon held vertically.

40. **Ficalbia (Mimomyia) flavopicta** Edw.

Known only from Bwamba lowlands and from Kisumu and Kakamega, Kenya.

FEMALES. *Biting by day*—forest, very rare. *By night*—canopy, very rare.

41. **F. (M.) mimomyiaformis** (Newst.).

Lowlands only.

Females. *Not biting*—tents (on open ground), scarce.

42. **F. (M.) plumosa** (Theo.).

Lowlands only.

Females. *Biting by night*—open ground and huts, very rare. *Not biting*—forest, occasional.

Variation. Third hind-tarsal segment rather dark, only the distal third white.

43. **F. (Etorleptiomyia) mediolineata** (Theo.).

Lowlands only.

Females. *Biting by night*—open ground and huts, scarce; forest and plantations, very rare. *Not biting*—huts and tents, common locally; forest, scarce.

Variation. Most of head scales yellow, but some upright black scales on nape, and palpi with some yellow scales at base above. Hind femora largely dark anteriorly, but with two well-defined yellow spots on apical half and sometimes extensively yellow in front; hind tibiae consistently with a patch of yellow scales on anterior surface near tip.

44. **F. (E.) xanthozona** van S.

Known from Bwamba lowlands only.

Females. *Not biting*—forest, very rare.

Note on Probably Undescribed Species. A single male, resembling *F.* (*Ficalbia*) *nigra* (Theo.), but with certain differences, has been taken in lowland forest. Another single male, quite obviously a new species of the subgenus *Ficalbia*, was taken in the same habitat, the specimen being lost. This second mosquito was of a light buff colour throughout, with a strikingly clubbed proboscis.

45. **Taeniorhynchus (Coquillettidia) metallicus** (Theo.).

Lowlands only.

Females. *Biting by day*—forest and plantations, occasional. *By night*—canopy, scarce. *Not biting*—plantations, scarce.

Males. Forest and plantations, scarce.

46. **T. (C.) pseudoconopas** (Theo.).

Lowlands only. Not known in East Africa outside Uganda.

Females. *Biting by day*—forest, not uncommon; plantations, occasional. *By night*—forest, not uncommon; canopy, occasional.

Males. Forest, scarce.

Variation. Some characters given by Edwards (1941) as distinguishing this species from *T.* (*C.*) *maculipennis* (Theo.) have not proved satisfactory. Dark markings on anterior part of scutum may be almost entirely absent, and dark scales on wings may form distinct bands, as in *T. maculipennis*. A character which we have found useful is that in *T. maculipennis* the anterior half of the scutum has a distinctly burnished appearance, which does not seem to occur in *T. pseudoconopas*. Gillett (1946) has found good differences between the larvae.

47. **T. (C.) maculipennis** (Theo.).

Lowlands only.

Females. *Biting by day*—forest, not uncommon; canopy and plantations, occasional. *By night*—forest, common ; canopy, occasional. *Not biting*—forest and plantations, scarce.

MALES. Forest and plantations, occasional.

48. **T. (C.) fuscopennatus** Theo.

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, common ; canopy and open ground, occasional. *By night*—forest, huts and tents, very common ; canopy, plantations and open ground, common. *Not biting*—forest, plantations, huts and tents, common.

MALES. Forest and plantations, not uncommon.

VARIATION. Bwamba specimens are consistently lighter in colour than the very dark form common on the shores of Lake Victoria.

NOTE. This species has been taken biting monkeys, dogs and fowls as well as man.

49. **T. (C.) aurites** Theo.

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, not uncommon ; canopy, occasional. *By night*—canopy, common ; forest and plantations, not uncommon ; huts and tents, occasional. *Not biting*—huts and tents, scarce.

MALES. Plantations, scarce.

VARIATION. Hind legs with distal dark band on first tarsal segment often more extensive than indicated by Edwards (1941)—usually a quarter and sometimes one-third dark distally ; segments 3–5 of fore and hind tarsi usually all black, 3 occasionally with an indistinct narrow yellow band at base. Abdomen often with no black scales on tergites. See also Gillett (1949).

NOTE. As noted in the introduction, the position of the golden *Coquillettidia* spp. of the *aurites-aureus-microannulatus* group seems to be doubtful. Intergrades do occur (at least in so far as adult coloration is concerned) and though the styles appear quite distinct in Edwards' (1941) figures, it should be noted that a slight degree of rotation or distortion changes their appearance very markedly.

50. **T. (C.) fraseri** (Theo.).

Lowlands only. Owing to confusion between this species and *T.* (*C.*) *aurites* (see Gillett, 1949), detailed records of occurrence in Bwamba cannot at present be given, though *T.* (*C.*) *fraseri* is known to occur in the lowlands.

51. **T. (C.) aureus** Edw.

Lowlands only.

FEMALES. *Biting by day*—forest, occasional. *By night*—canopy, rare.

VARIATION. Abdomen and wing darker than usual ; numerous scattered dark scales on wing, including some on apical half of costa ; tergites II–IV with fairly large apical median patches of dark scales. Hind-tarsal segments 3 and 4 yellow on basal half.

52. **T. (C.) microannulatus** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, occasional. *By night*—canopy, rare.

MALES. Forest, very rare.

53. **T. (Mansonioides) africanus** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—forest, common ; plantations, huts and tents, not uncommon ; canopy and open ground, occasional. *By night*—forest, huts and tents, abundant ; canopy and plantations, very common ; open ground, not uncommon. *Not biting*—huts and tents, common ; plantations, occasional.

VARIATION. It may be noted that *T. africanus* from certain parts of Uganda (notably the shores of Lake Victoria in localities such as Entebbe and Sango Bay) are consistently darker than Bwamba specimens.

54. **T. (M.) africanus** var. **nigerrimus** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—forest, occasional. *By night*—forest and tents, occasional.

NOTE. Most of the specimens were taken near the Semliki River.

55. **T. (M.) uniformis** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—forest, huts and tents, common ; plantations, not uncommon ; canopy and open ground, occasional. *By night*—forest, huts and tents, very common ; canopy, plantations and open ground, not uncommon. *Not biting*—plantations, occasional.

VARIATION. As in the case of *T. africanus*, Bwamba specimens are consistently lighter in general colour than those from Lake Victoria, and the green bands on the thorax are often poorly defined.

NOTE. This species has been taken biting monkeys.

NOTE ON A POSSIBLE NEW SPECIES. A few males and females of a species closely resembling *T. microannulatus*, but differing in details of terminalic structure, have been taken in the lowland forest.

56. **Aëdes (Mucidus) scatophagoides** (Theo.).

Lowlands only.

FEMALES. *Biting by night*—canopy, occasional. *Not biting*—huts, rare.

57. **A. (M.) mucidus** (Karsch).

Lowlands only.

FEMALES. *Biting*—canopy, very rare (only one taken).

58. **A. (M.) nigerrimus** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—forest and canopy, occasional. *By night*—canopy, common ; forest, occasional.

LARVAE. Ground-pools in forest, rare.

VARIATION. Fore and sometimes mid tarsi with white basal band on second segment absent ; hind tibiae with a distinct white patch above at middle. Wings sometimes with definite but small white areas at base of costa.

59. **A. (M.) grahami** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—canopy, occasional ; forest, rare. *By night*—canopy, common ; forest and plantations, occasional.

VARIATION. White basal band on second fore-tarsal segment sometimes absent. Wing with costa and first vein extensively yellow on apical third.

NOTE. This species has been taken biting monkeys.

60. **A.** (**Finlaya**) **longipalpis** (Grünb.).

Lowlands only.

FEMALES. *Biting by day*—canopy, very common ; forest, occasional ; plantations, scarce. *By night*—canopy, occasional.

MALES. Reared material only.

LARVAE AND PUPAE. Tree-holes in forest, very common.

VARIATION. Fore legs not entirely dark—fore tarsi being marked as mid and hind legs with a narrow white basal band on first segment, and second usually one-half to two-thirds white basally ; sometimes white markings reduced to small white dorsal spots. Otherwise as described by Edwards (1941) but mid femora with anterior median silvery patch sometimes extending back as a narrow stripe below to near base, fore and mid tibiae with narrow white basal bands extended a short distance below, and hind tibiae with white basal area below extended upward to form a broad or narrow band.

NOTE. This species has been taken biting monkeys.

61. **A.** (**F.**) **ingrami** Edw.

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, not uncommon ; canopy, occasional. *By night*—forest and canopy, occasional.

LARVAE AND PUPAE. Tree-holes in forest, not uncommon.

VARIATION. Adult ornamentation very variable (van Someren, 1947). Additional characters noted are : fore tarsi occasionally all dark and second segments of mid and hind tarsi often wholly white above (though usually dark on distal half below). Scutum sometimes very dark with white areas reduced to narrow lines, especially on posterior half.

NOTE. This species has been taken biting monkeys.

62. **A.** (**F.**) **pulchrithorax** Edw.

Known only from Bwamba highlands and Kenya highlands.

MALES AND FEMALES. Reared material only.

LARVAE AND PUPAE. Tree-holes and bored bamboos in mountain forest, not uncommon.

VARIATION. Larvae with head seta C 2–4 branched.

63. **A.** (**Stegomyia**) **aegypti** (L.).

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, scarce. *By night*—canopy, occasional ; forest, rare.

MALES. Reared material only.

LARVAE AND PUPAE. Receptacles in plantations, common ; receptacles in and around huts, not uncommon ; tree-holes in forest and plant-axils in plantations, occasional.

64. **A.** (**S.**) **simpsoni** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—plantations, very common ; forest, canopy and open ground, occasional. *By night*—forest, canopy and plantations, occasional. *Not biting*—plantations, common ; huts and tents, exceptional.

MALES. Plantations, common.

LARVAE AND PUPAE. Plant-axils in plantations, abundant ; receptacles in plantations, occasional ; tree-holes in forest and receptacle in house, exceptional.

VARIATION. Markings variable, all distinguishing characters noted by Edwards (1941) for *A.* (*S.*) *metallicus* (Edw.) having been seen in this species, except the broad metallic scales round bare space. Claws of female (in small sample examined for this character) toothed. Median yellow lines on scutum variable, sometimes absent, often not reaching beyond the half, but frequently continued forward to front margin. Very occasionally a line of white scales round anterior edge of bare space joins the two median lines (at a glance specimens of this type might be mistaken for *A. metallicus*). Abdominal tergites II and III often without basal white band.

NOTE. This species has been taken biting monkeys, goats and fowls.

Forest records, and more particularly those for the canopy, refer only to the extreme edges, and then only when in the neighbourhood of plantations. Records for open ground refer also to the close vicinity of plantations.

65. **A.** (**S.**) **simpsoni** var. **lilii** (Theo.).

Lowlands only.

MALES AND FEMALES. As above.

NOTE. In view of the marked variability of *A. simpsoni*, it seems obvious that the colour characters for this variety fall within the normal range of variation. The inconstant claw character clearly requires further investigation.

66. **A.** (**S.**) **apicoargenteus** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—canopy, very common ; forest, not uncommon ; plantations, occasional. *By night*—forest and canopy, occasional.

MALES. Reared material only.

LARVAE AND PUPAE. Tree-holes in forest, very common ; receptacles in plantations, exceptional.

VARIATION. Amount of white on posterior surface of hind femora varies from a very narrow basal band to basal half white (usually about one-third is white), anterior surface sometimes covered with silvery scales on basal three-fifths. Lateral lobes of scutellum sometimes with silvery scales. Abdominal tergites III and IV sometimes (and II occasionally) with a pair of small median basal spots ; those on IV often large.

NOTE. This species has been taken biting monkeys.

67. **A.** (**S.**) **fraseri** (Edw.).

Lowlands only.

FEMALES. *Biting by day*—forest and canopy, not uncommon.

MALES. Forest, rare.

LARVAE AND PUPAE. Tree-holes in forest, scarce.

VARIATION. As noted elsewhere (van Someren, 1946a) adult ornamentation is variable, especially size of lateral white spots on scutum, and amount of white on hind femora. Bwamba specimens have lateral white scutal spots large and hind femora white all round on basal third, with posterior surface black on distal two-thirds and white median patch on anterior surface running back to meet basal white area.

68. **A. (S.) dendrophilus** Edw.

Lowlands only.

FEMALES. *Biting by day*—forest, not uncommon ; canopy and plantations, occasional. *By night*—forest, occasional ; canopy, rare. *Not biting*—forest, not uncommon.

MALES. Forest, not uncommon ; plantations, occasional.

LARVAE AND PUPAE. Tree-holes in forest, common.

VARIATION. Adult ornamentation very variable, especially extent of white markings on scutum and tarsi (van Someren, 1946a).

NOTE. Owing to errors in published literature, this mosquito was for a time referred by the writers to *A.* (*S.*) *de-boeri* subsp. *de-meilloni* Edw. (van Someren, 1946a ; Haddow & others, 1947).

69. **A. (S.) africanus** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—forest and canopy, not uncommon ; plantations, occasional. *By night*—canopy, very common ; forest, not uncommon ; plantations, occasional. *Not biting*—forest, not uncommon.

MALES. Reared material only.

LARVAE AND PUPAE. Tree-holes in forest, very common ; receptacles around huts, exceptional.

VARIATION. Markings exceedingly variable. Either head, thorax, or occasionally both together, may approximate quite closely in appearance to *A.* (*S.*) *luteocephalus* (Newst.). Median line on scutum particularly variable, usually not extending full length of scutum. One or two specimens with banded abdomen have been taken, the bands being white or buff (these specimens were otherwise typical *A. africanus*). Occasionally fourth hind-tarsal segment has an exceptionally wide white band as in third. A large number of Bwamba larvae closely resemble the description and figures given by Hopkins (1936) for *A. luteocephalus*, adults reared out in such cases usually being quite typical *A. africanus*.

NOTE. This species has been taken biting monkeys, and there is a certain amount of evidence that they may be its preferred food.

A. africanus, together with *A. apicoargenteus*, is the only species of *Stegomyia* which we have taken in the forest interior, far from the road or the Semliki River.

70. **A. (S.) luteocephalus** (Newst.).

Lowlands only.

FEMALES. *Biting by day*—forest, rare. *By night*—canopy, occasional ; forest, rare.

NOTE. Forest records all refer to the extreme edges of forest, bordering on savannah.

The writers cannot give definite records of larvae, as many which have appeared to belong to this species have yielded typical *A. africanus* adults.

71. **A. (S.) ruwenzori** Haddow & van S.

Known only from Bwamba highlands and other parts of the Ruwenzori Mountains.

FEMALES. *Biting by day*—forest and canopy, occasional. *By night*—canopy, very common ; forest, occasional.

NOTE. At the time of writing the larva of this very distinctive species of the

A. africanus group has not yet been found, in spite of extensive surveys of tree-holes, bored bamboos, plant-axils and streams in the Ruwenzori mountain forests. Attempts to obtain eggs from captive females have also failed. On one occasion three larvae resembling that of *A. africanus* were found in a tree-hole in mountain forest. These were unfortunately in the first stage. It is possible that they may have belonged to this species.

72. **A. (S.) vittatus** (Big.).

Lowlands only.

FEMALES. *Biting by day*—open ground, very rare (only one taken).

73. **A. (Aëdimorphus) capensis** Edw.

Both lowlands and highlands.

FEMALES. *Biting by day*—forest and plantations, scarce. *By night*—forest, scarce.

LARVAE AND PUPAE. Tree-holes in forest, occasional.

VARIATION. All specimens of type A (Edwards, 1941).

74. **A. (A.) stokesi** Ev.

Lowlands only. Not known in East Africa outside Uganda.

FEMALES. *Biting by day*—forest and plantations, occasional. *By night*—forest, occasional ; canopy, rare.

MALES. Forest, very rare.

LARVAE AND PUPAE. Tree-holes in forest, occasional.

VARIATION. Adult ornamentation variable, especially that of scutum. Lateral silvery lines on scutum sometimes not reaching, but often extending to (and occasionally over), scutal angle ; these lines often broad in front and narrow behind, but sometimes same breadth throughout ; very occasionally scutum with two small spots of narrow yellow scales in middle. Fore tarsi sometimes with a small white spot above on second segment.

NOTE. In view of the variation discussed above, some specimens were for a time assigned to *A.* (*A.*) *haworthi* Edw. (Haddow & others, 1947).

75. **A. (A.) simulans** (Newst. & Cart.).

Not known in East Africa outside Bwamba lowlands.

FEMALES. *Biting by day*—forest and plantations, occasional. *By night*—forest, scarce.

MALES. Forest, rare.

LARVAE AND PUPAE. Tree-holes in forest, not uncommon.

VARIATION. Male terminalia as in Edwards' (1941) figure, except that apical portion of style seems slightly broader and three of the hairs at base of inner margin of coxite are longer than the others. All males have a pale patch on proboscis in middle below, but females often have proboscis all dark. Fore tarsi often with a white apical spot on segments 1 and 2, and under surfaces of all femora white, not buff ; hind femora often white to near tip below ; white pre-apical spot on anterior surface of hind femora not divided from white basal area, and more a patch extending on an upper surface than a spot. Larval comb-scales narrower than those figured by Hopkins (1936).

76. **A. (A.) argenteopunctatus** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, not uncommon. *By night*—

forest and plantations, occasional ; canopy, rare. *Not biting*—forest and plantations, not uncommon.

MALES. Forest, scarce.

77. **A. (A.) hopkinsi** Edw.

Lowlands only. Not known outside Uganda.

MALES. Forest, very rare (only one taken).

VARIATION. Male terminalia with coxite rather broader and basal lobe larger than in Edwards' (1941) figure. Scutum with broad lines of narrow white scales round bare space and two short lines of narrow white scales placed just beyond and slightly outside lines round bare space ; very few (2—4) white scales above wing root. Lateral white patches on tergites large, visible dorsally, and slightly removed from bases of segments.

78. **A. (A.) mutilus** Edw.

Lowlands only. Not known in East Africa outside Uganda.

FEMALES. *Biting by day*—forest, not uncommon ; plantations, occasional. *By night*—forest, occasional ; canopy, scarce. *Not biting*—forest, occasional.

MALES. Forest, rare.

LARVAE AND PUPAE. Forest ground-pools, rare.

VARIATION. Style of male terminalia with no (or very little) indication of a terminal horn. Tergites II–V sometimes with silvery basal bands. Larva very like that of *A.* (*A.*) *filicis* I. & De M., but with five (instead of three) pecten teeth large, simple and widely spaced (van Someren, 1950b).

79. **A. (A). domesticus** (Theo.).

Lowlands only. Not known in East Africa outside Uganda.

FEMALES. *Biting by day*—forest and plantations, not uncommon. *By night*—forest, scarce. *Not biting*—forest and plantations, occasional.

MALES. Forest, scarce.

VARIATION. Length of apical white spot on hind tibiae variable (from one-eighth to one-sixth length of tibiae). Scutum with silvery shoulder patches sometimes reduced to small spots.

80. **A. (A.) tarsalis** (Newst.).

Lowlands only.

MALES. Forest, not uncommon.

LARVAE AND PUPAE. Ground-pools in forest, common.

VARIATION. Hopkins (1936) states that the larva of *A. tarsalis* differs from that of *A. filicis* by having fewer branches (7–9) in head seta A and many more comb-scales (40–60). Bwamba larvae, checked by bred material, have head seta A with 12–14 branches and comb with only 20–30 scales. Subventral tuft just beyond one half.

NOTE. In view of the difficulty of distinguishing mosquitoes of the *A. tarsalis* group except by the examination of male terminalia, most of our records refer to the group rather than to *A. tarsalis* (*s. str.*). Group records are as follows :

FEMALES. *Biting by day*—forest, abundant ; plantations, common ; canopy and open ground, scarce. *By night*—forest and plantations, not uncommon ; canopy, open ground, huts and tents, scarce. *Not biting*—forest and plantations, not uncommon.

MALES. Forest, not uncommon ; plantations, occasional.

81. **A. (A.) phyllolabis** Edw.

Lowlands only.

Males. Forest, scarce.

Larvae and Pupae. Ground-pools in forest, occasional.

82. **A. (A.) albocephalus** (Theo.).

Lowlands only.

Males. Forest, canopy and plantations, occasional.

Variation. According to Edwards (1941) the presence of post-spiracular scales in both sexes and the banded abdomen of the female easily differentiates *A. albocephalus* from *A. tarsalis*. These characters are not reliable, as some male *A. albocephalus* from Bwamba have no post-spiracular scales, and females with post-spiracular scales often have no basal white bands on the tergites.

83. **A. (A.) smithburni** van S.

Known from Bwamba lowlands only.

Females. *Not biting*—forest, scarce.

Males. Forest, scarce.

84. **A. (A.) congolensis** Edw.

Lowlands only. Not known in East Africa outside Bwamba.

Males. Plantations, very rare (only 1 taken).

Note. As in the *A. tarsalis* group, great difficulties in identification characterise the *A. abnormalis* group (as defined by Edwards, 1941), male terminalia being the only reliable diagnostic character. As a result, most of our records refer to the group rather than to any particular species. These group records are as follows :

Females. *Biting by day*—forest, very common ; plantations, not uncommon ; canopy and open ground, scarce. *By night*—forest, not uncommon ; plantations, occasional ; canopy, scarce. *Not biting*—forest and plantations, occasional.

Males. Forest, occasional.

85. **A. (A.) tricholabis** subsp. **bwamba** van S.

Known from Bwamba lowlands only.

Females. *Biting by day*—forest, scarce. *Not biting*—forest, scarce.

Males. Forest and plantations, rare.

Larvae and Pupae. Reared from eggs laid by a captive female.

86. **A. (A.) semlikiensis** van S.

Known from Bwamba lowlands only.

Females. *Biting by day*—forest, occasional.

Males. Forest, scarce.

87. **A. (A.) abnormalis** subsp. **kabwachensis** Edw.

Known only from Bwamba lowlands and Kenya.

Males. Forest, occasional.

Variation. Scutum with a background of narrow dark scales, and with many scattered golden scales, sometimes forming a definite pattern ; scutellar scales pale, usually broad on lateral lobes and narrow on middle lobe, but sometimes a line of broad scales at base of middle lobe.

88. **A. (A.) wigglesworthi** Edw.

Not known in East Africa outside Bwamba lowlands.

MALES. Forest, rare.

VARIATION. Mainly as described by Edwards (1941), but terminal segments of male palpi not bare—numerous long hairs present below, and usually some flat scales above. Thorax sometimes with scales on *apn* broader than usual in this group and scales on paratergites narrow or broad.

89. **A. (A.) lamborni** Edw.

Lowlands only.

FEMALES. *Biting by day*—forest, scarce. *By night*—forest, rare ; canopy, very rare. *Not biting*—forest, rare.

VARIATION. Head with a large patch of narrow yellow scales on either side of vertex in addition to those on nape and around eyes (sometimes vertex almost entirely covered with yellow scales). Scutellum with the broad pale scales creamy white or pale yellow ; pale scales on sternites yellowish.

90. **A. (A.) dentatus** (Theo.).

Highlands only.

FEMALES. *Biting by day*—forest, common. *By night*—forest, not uncommon ; canopy, rare.

91. **A. (A.)** aff. **dentatus** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, occasional. *By night*—forest, occasional.

NOTE. Females of this species closely resemble *A. dentatus*. In view, however, of the unlikely habitat in lowland rain-forest and plantations, we hesitate to assign them finally to this species. It is possible that they may belong to a new species or subspecies of the *A. dentatus* group, but final decision must await the examination of males.

92. **A. (A.) cumminsi** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, very common ; open ground, occasional ; canopy, rare. *By night*—forest and plantations, very common ; open ground, huts and tents, occasional ; canopy, rare. *Not biting*—forest and plantations, common ; huts and tents, occasional.

MALES. Forest and plantations, not uncommon.

LARVAE AND PUPAE. Ground-pools in forest, common.

NOTE. In view of the fact that both the named subspecies of *A. cumminsi* (though rare) occur in Bwamba together with the typical form, they cannot properly be regarded as subspecies. It is therefore suggested here that they should be reduced to the rank of varieties. It might even be preferable to consider *A. cumminsi* as a variable species rather than to subdivide it into a series of named varieties.

93. **A. (A.) cumminsi** var. **mediopunctatus** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—forest, scarce.

94. **A. (A.) cumminsi** subsp. **holocinctus** Edw.

Lowlands only.

FEMALES. *Biting by day*—forest, scarce.

95. **A. (A.) pubescens** Edw.

Not known in East Africa outside Bwamba lowlands.

FEMALES. *Biting by day*—forest, scarce.

MALES. Forest, scarce.

VARIATION. Scutum with many scattered golden scales in middle, and golden border sometimes quite broad.

96. **A. (A.) natronius** Edw.

Lowlands only.

FEMALES. *Biting by day*—forest and canopy, scarce. *By night*—canopy, not uncommon ; forest, scarce. *Not biting*—forest, scarce.

MALES. Forest, rare.

LARVAE AND PUPAE. Tepid saline pools and slow streams flowing from hot springs, not uncommon.

VARIATION. Abdominal markings variable. Females sometimes with all pale scales on tergites yellow, but usually the basal bands white ; tergites II–IV very often with no apical yellow markings, but sometimes with very small subapical lateral spots ; V–VII usually with lateral spots but occasionally with narrow yellow apical bands. Males with no yellow markings on tergites—basal whitish bands only, and perhaps a few pale scales on apical corners.

97. **A. (A.) ochraceus** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—plantations, very rare (only one taken).

98. **A. (Banksinella) circumluteolus** (Theo.).

Both lowlands and highlands.

FEMALES. *Biting by day*—forest and plantations, abundant ; open ground, not uncommon ; canopy, scarce. *By night*—forest and plantations, very common ; open ground, occasional ; canopy, huts and tents, scarce.

MALES. Forest and plantations, not uncommon.

VARIATION. Tori very often all dark. Scutum usually with a dark area in middle and two broad or narrow yellow median lines running the full length (but sometimes incomplete or absent) ; sometimes middle of scutum covered with yellow scales not much darker than those of lateral lines. Tergites very occasionally mottled with yellow and dark scales ; male sternites all dark and sternites of female usually yellow (but sometimes mottled with light and dark scales, and occasionally all dark). Legs without a yellow spot at tip of hind tibiae.

99. **A. (B.) albothorax** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, not uncommon. *By night*—forest and plantations, occasional.

MALES. Forest, scarce.

VARIATION. Proboscis with pale patch in middle ; below often very faint. Pale scales on scutum light yellow or creamy white but not definitely white or greyish ;

pattern sometimes very like that described above for *A. circumluteolus* but scutum more often entirely covered with pale scales. Tergites usually with basal lateral yellow patches only, but sometimes with apical yellow patches also ; no connecting yellow lines seen. One specimen with apical third of costa pale but not definitely white. Male terminalia with at least 10 spines on distal portion of coxite.

100. **A. (B.) taeniarostris** (Theo.).

Not known in East Africa outside Bwamba lowlands.

FEMALES. *Biting by day*—forest and plantations, occasional. *By night*—plantations, scarce. *Not biting*—forest, scarce.

MALES. Forest, scarce.

VARIATION. Females often with yellow band in middle of proboscis broadly interrupted above. Scutum with the two median yellow lines very distinct. Yellow patch on hind tibiae sometimes represented only by a few scattered scales ; middle tibiae sometimes without a yellow spot at tip above. Wings very often with no yellow scales on fifth vein. Males with no yellow patches on proboscis or hind tibiae.

101. **A. (B.) palpalis** (Newst.).

Lowlands only.

FEMALES. *Biting by day*—forest, abundant ; plantations, common ; open ground, occasional ; canopy, rare. *By night*—forest, very common ; canopy, rare. *Not biting*—forest, very common ; plantations, common.

MALES. Forest and plantations, not uncommon.

VARIATION. Scutum often with the median yellow lines distinct. Middle tibiae sometimes with a yellow apical spot above. A few specimens have the yellow scales on scutum very pale, almost white.

102. **A. (B.) carteri** Edw.

Not known in East Africa outside Bwamba lowlands.

FEMALES. *Biting by day*—forest, occasional. *By night*—forest, occasional.

MALES. Forest, scarce.

VARIATION. Males have no pale patch on proboscis below nor on hind tibiae above. Females usually have a yellow patch on hind tibiae in middle above, but this is sometimes missing or represented only by a few scales forming a long or short ragged line ; proboscis usually all dark, sometimes with an indistinct (occasionally very distinct) yellow ventral patch which extends up the sides.

The extreme variation in the yellow markings of this mosquito makes it difficult to determine females with complete certainty, as dark specimens might be *A. palpalis* and paler forms could be *A. taeniarostris*.

NOTE. In view of the fact that the distribution of this mosquito overlaps that of *A. palpalis* in Bwamba, it cannot properly be regarded as a subspecies of *A. palpalis* (as suggested by Edwards, 1941). We consider that, as proposed here, it should be raised to full specific rank.

103. **A. (Diceromyia) furcifer** (Edw.).

Lowlands only.

FEMALES. *Biting by night*—canopy, not uncommon.

MALES. Forest, scarce.

VARIATION. Female proboscis occasionally without a definite white ring, but with numerous scattered pale scales on middle third.

NOTE. Three males have so far been taken and dissected. All agree with

Edwards' (1941) description of the hypopygial characters of *furcifer* (Edw.) ; all records are therefore included here.

104. **A. (Dunnius) albomarginatus** (Newst.).

Lowlands only.

FEMALES. *Biting by day*—forest, occasional ; plantations, scarce. *By night*—forest, scarce. *Not biting*—forest, occasional.

MALES. Forest, very rare.

VARIATION. As mentioned elsewhere (van Someren, 1947) the pale posterior lateral lines on the scutum vary in length. Additional characters noted are : pale posterior lines may be pure white or (occasionally) absent ; sternite VII may be nearly all white, or with a broad (or narrow) basal white band.

NOTE. This mosquito was at first taken to be a new species (Haddow, 1945b).

NOTE ON PROBABLY UNDESCRIBED SPECIES. Two species, almost certainly new, occur in the subgenus *Banksinella.* The first resembles *A. bolensis* Edw., the thorax being almost completely and uniformly golden above. Only one female has so far been taken, biting in forest by day. The second, which belongs to the *A. taeniarostris* group, has the hind tibiae all dark, and the proboscis with a large ventral patch of pale scales in the middle, often extending up the sides so that it almost forms a band. Only females, biting in forest by day, have so far been taken. An undescribed larva, belonging to the subgenus *Aëdimorphus* occurs in ground-pools, and is characterised by a patch of long simple spines on the dorsal surface of the siphon just before the tip.

105. **Eretmapodites chrysogaster** Graham.

Lowlands only.

FEMALES. Reared material only.

MALES. Forest and plantations, very common.

LARVAE AND PUPAE. Leaf-pools in forest and plantations, very common ; plant-axils in plantations, common ; receptacles in forest and plantations, not uncommon ; tree-holes in forest, occasional ; receptacles in open ground and ground-pools in forest, exceptional.

NOTE. In view of the impossibility of distinguishing females of this group reliably, the above records are confined to males and to larvae and pupae from which males have been reared out. Records for the *E. chrysogaster* group are as follows :

FEMALES. *Biting by day*—forest and plantations, very common ; open ground, occasional. *By night*—forest and plantations, occasional ; canopy, scarce. *Not biting*—forest and plantations, common.

MALES. Forest and plantations, common.

LARVAE AND PUPAE. Leaf-pools in forest and plantations, abundant ; plant-axils in plantations, very common ; tree-holes in forest, occasional.

NOTE. Variation in *Eretmapodites* spp. has been discussed by Haddow (1946) and van Someren (1949). The predatory habits of the larvae have been described by Haddow (*loc. cit.*).

106. **E. intermedius** Edw.

Lowlands only.

MALES. Forest, occasional.

107. **E. mahaffyi** van S.

Known from Bwamba lowlands only.

MALES. Forest, very rare (only one taken).

108. **E. haddowi** van S.

Known from Bwamba lowlands only.

MALES. Forest, very rare (only three taken).

109. **E. harperi** van S.

Known from Bwamba lowlands only.

MALES. Forest and plantations, rare (12 taken).

110. **E. semisimplicipes** Edw.

Lowlands only.

FEMALES. Reared material only.

MALES. Forest and plantations, not uncommon.

LARVAE AND PUPAE. Leaf-pools in forest and plantations, very common ; plant-axils in plantations, common.

NOTE. As mentioned elsewhere (Haddow, 1946) females of this species have the scutal scaling much darker than other members of the group. It is not yet known how reliable this character will prove. If it be accepted for the time being, the following records may be added :—

FEMALES. *Biting by day*—forest and plantations, common. *By night*—forest, scarce. *Not biting*—forest and plantations, common.

111. **E. gilletti** van S.

Known from Bwamba lowlands only.

MALES. Forest, rare (ten taken).

112. **E. grahami** Edw.

Not known in East Africa outside Bwamba lowlands.

FEMALES. Reared material only.

MALES. Forest and plantations, not uncommon.

LARVAE AND PUPAE. Snail-shell in forest (once only).

NOTE. Some females, lacking a pale line on the proboscis, were taken biting in forest and plantations by day, and resting females of this type were taken in the same habitats. These were originally assigned to this species (Haddow, 1946) but examination of reared material has shown that this character is not reliable, at least in females.

113. **E. inornatus** Newst.

Not known in East Africa outside Bwamba lowlands.

FEMALES. *Biting by day*—forest and plantations, occasional. *Not biting*—forest and plantations, occasional.

MALES. Forest and plantations, rare.

LARVAE AND PUPAE. Snail-shells in forest, not uncommon ; plant-axils in plantations and leaf-pools in forest, rare.

NOTE. The only good diagnostic character found among the females of this group is the form of the fore claws (Haddow, 1946). The above records of females (and the similar records for *E. penicillatus* Edw.) refer to specimens in which the claws have been examined. The more extensive group records are as follows :—

FEMALES. *Biting by day*—forest and plantations, not uncommon. *By night*—forest, occasional ; canopy, very rare. *Not biting*—forest and plantations, not uncommon.

MALES, LARVAE AND PUPAE. As above.

114. **E. penicillatus** Edw.

Not known in East Africa outside Bwamba lowlands.

FEMALES. *Biting by day*—forest and plantations, occasional. *Not biting*—forest and plantations, occasional.

MALES. Forest, scarce.

LARVAE AND PUPAE. Snail-shells in forest, not uncommon.

115. **E. tonsus** Edw.

Known only from Bwamba lowlands and from Kakamega, Kenya.

MALES. Forest, very rare (only one taken).

VARIATION. The scutum is slightly rubbed, but is as described by Edwards (1941), patterned as in *E. inornatus* with narrow yellow lines. Additional characters noted are :

Middle lobe of scutellum with broad black scales only, lateral lobes with narrow yellow scales ; *apn* (not *ppn* as stated by Edwards) with silvery scales ; tergite I with two small dorsal silvery spots, and II with the oblique silvery lateral line reaching only half way to posterior margin ; posterior surface of mid and hind femora yellow on about basal two-thirds, a few white scales above at tip of mid femora, and a narrow white band at tip of hind femora.

116. **E. quinquevittatus** Theo.

Lowlands only.

MALES. Forest and plantations, very rare (only two taken).

VARIATION. Both specimens very small ; one badly rubbed ; yellow lines on scutum of other narrower than usual, though not as narrow as in the *E. inornatus* group ; sublateral yellow lines short, reaching only to middle of scutum.

NOTE. In a previous communication (Haddow, 1946) it was suggested that Gibbins' (1942) record of *E. quinquevittatus* from Bwamba should be referred to *E. inornatus*. Gibbins' record was based on pupae, and the pupae of these two species are separable with difficulty, if at all. Since that time, males of *E. quinquevittatus* have been taken, and it is no longer possible to say to which species Gibbins' material belonged. Probably, however, Gibbins' record should still be referred to *E. inornatus*, as his specimens came from an area in which we have collected intensively for six years and from which we have never taken *E. quinquevittatus*, though *E. inornatus* is relatively common.

117. **E. ferox** Haddow.

Known from Bwamba lowlands only.

FEMALES. *Biting by day*—forest, very common ; plantations, common. *By night*—forest and plantations, scarce. *Not biting*—forest and plantations, not uncommon.

MALES. Forest and plantations, not uncommon.

LARVAE AND PUPAE. Plant-axils in plantations, common ; leaf-pools in forest and plantations, not uncommon.

NOTE. When this mosquito was first described (Haddow, 1946) the male terminalia were thought to differ quite strikingly from those of *E. dracaenae* Edw. Since that time, however, we have examined the terminalia of the type male of *E. dracaenae*, and have found that the description and figure given by Edwards (1941) are both incomplete and misleading. The terminalia of the two mosquitoes are very similar, and *E. dracaenae* possesses the harpoon-like sigmoid hairs, digitate hairs, and spoon-shaped scales on the basal lobe which had been supposed to be characteristic of *E. ferox* exclusively. Further, the difference in the shape of the basal lobe of the two mosquitoes is obviously due to the orientation of the specimens. The sole remaining difference in the terminalia is that in *E. dracaenae* the distal claspette bears numerous short hairs with enlarged tips, whereas in *E. ferox* these are simple.

118. **E. oedipodius** subsp. **parvipluma** Edw.

Lowlands only.

FEMALES. *Biting by day*—forest, occasional.

MALES. Forest, occasional.

LARVAE AND PUPAE. Leaf-pools in forest, scarce.

NOTE. The subspecies *parvipluma* is the Uganda lowland form, extending south-westward to Lake Kivu. It is surprising that it should also occur in Bwamba, where the Congo forest form (subsp. *stanleyi* Edw.) would have been expected.

119. **E. leucopus** subsp. **productus** Edw.

Lowlands only. Known elsewhere in East Africa only from Kaimosi, Kenya.

FEMALES. *Biting by day*—forest, not uncommon ; plantations, occasional. *By night*—forest, scarce. *Not biting*—forest and plantations, occasional.

MALES. Forest, scarce.

LARVAE AND PUPAE. Plant-axils in plantations and leaf-pools in forest and plantations, not uncommon ; tree-holes in forest, rare.

120. **Culex** (**Lutzia**) **tigripes** Grp. & Charm.

Lowlands only.

FEMALES. *Biting by day*—forest, not uncommon ; plantations, occasional. *By night*—forest, huts and tents, occasional ; canopy, rare. *Not biting*—forest, occasional.

MALES. Forest and plantations, scarce.

LARVAE AND PUPAE. Ground-pools in forest, not uncommon ; tree-holes in forest, rare.

VARIATION. Edwards (1941) gives five colour variations for this mosquito. Bwamba specimens resemble the third colour form (var. *mombasaensis*) but the apical dark markings on the sternites are distinctly interrupted in the middle.

121. **C.** (**Neoculex**) **kingianus** Edw.

Lowlands only.

FEMALES. *Not biting*—forest, rare.

MALES. Forest, rare.

122. **C.** (**N.**) **kilara** van S.

Known from Bwamba lowlands only.

FEMALES. *Not biting*—forest, rare.

MALES. Forest, very rare (only eight taken).

123. **C.** (**N.**) **rubinotus** Theo.

Lowlands only.

FEMALES. *Not biting*—forest, scarce.

MALES. Forest, scarce.

124. **C.** (**N.**) **insignis** (Cart.).

Lowlands only.

FEMALES. *Not biting*—forest, not uncommon.

MALES. Forest, not uncommon.

125. **C.** (**N.**) **wigglesworthi** Edw.

Lowlands only.

MALES. Above forest canopy, very rare (only one taken).

126. **C.** (**N.**) **albiventris** Edw.

Lowlands only.

FEMALES. *Not biting*—forest, occasional.

MALES. Forest and plantations, occasional.

LARVAE AND PUPAE. Tree-holes in forest, not uncommon.

VARIATION. Female occasionally with pale grey instead of black scales on sternite VII, and one male with small apical white spots on tergite VII.

127. **C.** (**N.**) **kanyamwerima** van S.

Known from Bwamba lowlands only.

MALES. Forest, very rare (only one taken).

128. **C.** (**N.**) **horridus** Edw.

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, occasional. *By night*—forest and plantations, scarce. *Not biting*—forest and plantations, not uncommon.

MALES. Forest, occasional.

129. **C.** (**Culiciomyia**) **nebulosus** Theo.

Both lowlands and highlands.

FEMALES. *Not biting*—forest, not uncommon.

MALES. Forest and plantations, not uncommon.

LARVAE AND PUPAE. Tree-holes in forest and tree-holes and bored bamboos in mountain forest, very common ; tree-holes, plant-axils and receptacles in plantations, common ; leaf-pools in forest and plantations, receptacles in huts and ground-pools in forest and plantations, occasional ; native pit-latrine, exceptional.

130. **C.** (**C.**) **cinereus** Theo.

Lowlands only.

FEMALES. *Not biting*—forest, scarce.

MALES. Forest, scarce.

VARIATION. Both males and females with only a small patch of scales in middle of mesepimeron (this patch occasionally absent) ; other scale-patches on pleuron also reduced. Lateral pale areas on tergites sometimes extending full length, or present on apical corners only.

131. **C.** (**C.**) **cinerellus** Edw.

Lowlands only. Not known in East Africa outside Uganda.

MALES. Forest and plantations, not uncommon.

LARVAE AND PUPAE. Tree-holes in forest, scarce.

VARIATION. Colouring of pleuron very variable—pale green or yellowish with dark lines across middle, and none with the upper half darker than the lower.

132. **C. (C.) semibrunneus** Edw.

Lowlands only. Not known in East Africa outside Uganda.

MALES. Forest, scarce.

VARIATION. Pleural integument uniform mid-brown or yellowish, faintly darker above, and with a slight grey dusting.

133. **C. (C.) subaequalis** Edw.

Lowlands only.

MALES. Forest, not uncommon ; plantations, scarce ; canopy, rare.

VARIATION. Pleural integument pale green, yellow or brown with or without two dark stripes. Male sometimes with only five outstanding scales on palps, and terminalia with basal arm or paraprocts sometimes shorter than those figured by Edwards (1941).

NOTE. Owing to the very variable colour patterns of the pleural integument in *Culiciomyia* spp., the colour character used by Edwards (1941) for separating *C. cinerellus*, *C. subaequalis* and *C. semibrunneus* is unreliable. Females of these species are indistinguishable on external characters. Group records are as follows :

FEMALES. *Not biting*—forest, scarce.

134. **C. (C.) mongiro** van S.

Known from Bwamba lowlands only.

FEMALES. *Not biting*—forest, rare.

MALES. Forest, rare.

135. **C. (C.) macfiei** Edw.

Lowlands only. Not known in East Africa outside Uganda.

FEMALES. *Biting by day*—forest, occasional.

MALES. Forest, occasional.

LARVAE AND PUPAE. Tree-holes in forest, not uncommon.

VARIATION. Females sometimes with scales and hairs on lower mesepimeron ; male palpi with 12–13 outstanding scales on shaft.

136. **C. (Culex) poicilipes** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, occasional. *By night*—forest, occasional ; canopy, rare.

137. **C. (C.) annulioris** Theo.

Lowlands only.

FEMALES. *Biting by day*—forest, occasional. *By night*—forest, occasional. *Not biting*—forest, scarce.

VARIATION. Median pale spots on tergites very small.

138. **C. (C.) duttoni** Theo.

Lowlands only.

LARVAE AND PUPAE. Tree-holes in forest, leaf-pools in plantations and ground-pools in forest, occasional ; receptacles in plantations, exceptional.

139. **C. (C.) watti** Edw.

Lowlands only.

FEMALES. *Biting by day*—forest, scarce. *Not biting*—forest, scarce.

MALES. Forest, rare.

VARIATION. Ventral pale area in middle of proboscis occasionally extended upward to form a complete band ; no scales on tori and no white scales on palpi. Scutal scales of female mostly yellowish, but with two patches of dark scales on either side anteriorly and two short dark lines posteriorly ; males with scutal scaling mostly dark brown (but with numerous scattered yellow scales). Dark markings of pleuron not very distinct in some lights ; occasionally a few narrow scales present on post-spiracular area. Basal pale bands on tergites broad in males but often very narrow in females ; sternites sometimes with complete apical dark bands. Legs with posterior surfaces largely ochreous ; tarsi sometimes with (but usually without) very indistinct pale rings at the joints.

140. **C. (C.) argenteopunctatus** subsp. **kingi** (Theo.).

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, not uncommon. *By night*—forest and plantations, occasional. *Not biting*—forest and plantations, occasional.

MALES. Forest, rare.

VARIATION. Head often with a patch of dark scales in middle. Scutum with scales round bare space yellow, not white ; one specimen with the two lateral silvery spots on one side connected by a broad band of narrow silvery scales ; pleural integument not paler than scutum.

141. **C. (C.) univittatus** Theo.

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, occasional. *By night*—huts and tents, scarce. *Not biting*—forest, occasional.

MALES. Forest and plantations, occasional.

LARVAE AND PUPAE. Ground-pools in forest, not uncommon.

NOTE. The occurrence of the typical form together with the var. *neavei* Theo. in the same area is of interest.

142. **C. (C.) univittatus** var. **neavei** Theo.

Lowlands only.

MALES. Forest and plantations, not uncommon.

VARIATION. Very like Lake Albert form (described by Edwards, 1941, under variations). Sternites with some dark markings, either apical lateral dark spots or complete dark apical bands ; occasionally dark with pale lateral borders, or pale with a dark median stripe and apical band. Post-spiracular area usually with a few pale scales, but sometimes bare. Hind tibiae sometimes with a faint pale stripe anteriorly, and a distinct pale spot at tip.

143. **C. (C.) pipiens** L.

Lowlands only.

LARVAE AND PUPAE. Tree-holes and leaf-pools in forest, scarce.

144. **C. (C.) fatigans** Wied.

Lowlands only.

FEMALES. *Not biting*—forest, scarce.

LARVAE AND PUPAE. Ground-pools in forest, scarce.

145. **C. (C.) andersoni** Edw.

Highlands only.

MALES AND FEMALES. Reared material only.

LARVAE AND PUPAE. Tree-holes in forest, not uncommon.

VARIATION. Larvae with fewer comb-scales than usual (about 50 instead of 60).

NOTE. It is interesting to record that all specimens from the mountain forest were typical *C. andersoni*. This particular forest is the type locality for *C. andersoni* subsp. *bwambanus* Edw. Oddly enough, we have found this subspecies in the lowland forest only.

146. **C. (C.) andersoni** subsp. **bwambanus** Edw.

Lowlands only. Not known in East Africa outside Bwamba.

MALES. Forest, rare.

VARIATION. Scutal scales all golden yellow ; hind femora usually with anterior surface dark on distal half but sometimes appearing dark for full length, with basal half only indistinctly paler. Male palpi with very few hairs on distal segments.

NOTE. Owing to the entirely pale-scaled scutum these specimens would run down in Edwards' (1941) key to *C. hopkinsi* Edw. They differ from this species by having very few hairs on the terminal segments of the palpi. The terminalia, also, are more like those of subsp. *bwambanus*, having the style slender and the lobe of the coxite poorly developed.

147. **C. (C.) musarum** Edw.

Highlands only.

LARVAE AND PUPAE. Plant-axils in forest (wild banana) and in plantations, abundant.

148. **C. (C.) antennatus** (Beck.).

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, scarce.

LARVAE AND PUPAE. Ground-pools in forest, occasional.

149. **C. (C.) quasiguiarti** Theo.

Lowlands only.

MALES. Forest and plantations, occasional.

VARIATION. Male tergites with broad or narrow pale basal bands. Anterior surfaces of hind femora dark on distal eighth to sixth.

NOTE. It is extremely difficult to distinguish the females of *C. quasiguiarti* from the following three species, as all may have reddish-tinted scutal scales and as the extent of the apical dark area on the anterior surface of hind femora is variable. Grouped records for the four species are as follows :

FEMALES. *Biting by day*—forest and plantations, common. *Not biting*—forest and plantations, common.

150. **C. (C.) decens** Theo.

Lowlands only.

LARVAE AND PUPAE. Tree-holes in forest, leaf-pools in forest and plantations and ground-pools in forest, not uncommon.

151. **C. (C.) invidiosus** var. **vexillatus** Edw.

Lowlands only. Not known in East Africa outside Uganda.

MALES. Forest and plantations, occasional.

VARIATION. Scutal scales often with a reddish tint. Hind femora with dark dorsal line extending the full length (or only on distal two-thirds).

152. **C. (C.) trifoliatus** Edw.

Lowlands only. Not known in East Africa outside Uganda.

MALES. Forest and plantations, occasional.

VARIATION. Hind femora with distal sixth to fifth dark anteriorly, and pale areas sometimes clear white instead of creamy white ; pale spot at tip of hind tibiae sometimes absent.

153. **C. (C.) ornatothoracis** Theo.

Lowlands only. Not known in East Africa outside Uganda.

FEMALES. *Biting by day*—forest, scarce. *Not biting*—forest and plantations, occasional.

MALES. Forest and plantations, occasional.

VARIATION. Sometimes the two pale spots in middle of scutum absent. Anterior surface of hind femora dark on distal fourth to third ; pale spot at tip of hind tibiae not always very distinct.

154. **C. (C.) perfuscus** Edw.

Lowlands only.

FEMALES. *Not biting*—forest, occasional.

MALES. Forest and plantations, occasional.

LARVAE AND PUPAE. Ground-pools in forest, scarce.

VARIATION. All males differ from the description given by Edwards (1941) in having anterior surfaces of hind femora dark for full length, but females determined as this species are as in Edwards' description, with anterior surfaces white, except for a small dark spot at tip.

155. **C. (C.) guiarti** Blanch.

Lowlands only.

FEMALES. *Not biting*—forest, occasional.

MALES. Forest, occasional.

VARIATION. Scutum sometimes with all scales pale. Pleuron sometimes with a dark stripe across middle ; scale-patch in middle of mesepimeron small and in males often absent ; some males with two lower mesepimeral bristles. Knob of halteres sometimes dark.

156. **C. (C.) ingrami** Edw.

Lowlands only.

MALES. Forest, scarce.

LARVAE AND PUPAE. Ground-pools in forest, scarce.

VARIATION. Males often with no scale-patch in middle of mesepimeron, but sometimes a few scales present. Knob of halteres sometimes dark.

NOTE. It is extremely difficult to separate *C. guiarti* from *C. ingrami* on external characters. *C. guiarti* has a pale area in the scutal integument at the shoulders, and this has been used to separate it from *C. ingrami*, but it is doubtful if this is a reliable character.

157. **C. (C.) grahami** Theo.

Lowlands only.

MALES. Forest, scarce.

VARIATION. Scutal scales sometimes all pale creamy yellow. Knob of halteres sometimes dark. Male palpi longer than proboscis by terminal plus half penultimate segment.

158. **C. (C.) pruina** Theo.

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, common. *By night*—forest, huts and tents, occasional; canopy, rare. *Not biting*—forest and plantations, not uncommon.

MALES. Forest, not uncommon.

LARVAE AND PUPAE. Ground-pools in forest, not uncommon.

VARIATION. Female proboscis sometimes pale beneath in middle. Halteres sometimes dark. Fork cell of second vein from two and a half to three times length of stem.

NOTE. The larvae are of the var. *eschirasi* Galliard.

159. **C. (C.) pseudopruina** van S.

Known from Bwamba lowlands only.

MALES. Forest, scarce.

160. **C. (C.) moucheti** Ev.

Lowlands only.

FEMALES. *Biting by day*—forest and plantations, very common; open ground, occasional. *By night*—forest, not uncommon; canopy, rare. *Not biting*—forest and plantations, common.

MALES. Forest and plantations, common.

LARVAE AND PUPAE. Native pit-latrine.

VARIATION. Tori usually light orange, but sometimes very dark (almost brown). Legs largely yellow posteriorly.

NOTE. The peculiar breathing habits of the larva have been referred to by Gillett (1942).

NOTE ON PROBABLY UNDESCRIBED SPECIES. Among adults may be mentioned a mosquito resembling *C. pipiens* in colour and ornamentation, but with terminalia more closely resembling those of *C. antennatus*. Only one specimen has so far been taken, in lowland forest. From tree-holes in lowland forest, larvae have been taken which resemble those of *C.* (*N.*) *adersianus* Edw. The adult is so far unknown. In debris from plant-axils in plantations in the highlands the siphons of two unknown larvae have been recovered. One of these resembles that of *C.* (*C.*) *toroensis* Edw. & Gibbins, the other that of *C.* (*C.*) *trifilatus* Edw., but in both cases differences are present.

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THE SPATIAL DISTRIBUTION OF *CULICOIDES IMPUNCTATUS* GOET. UNDER WOODLAND AND MOORLAND CONDITIONS AND ITS FLIGHT RANGE THROUGH WOODLAND.

By D. S. Kettle, M.Sc. (Lond.).

(Plates X & XI.)

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Introduction.

In an earlier paper (Kettle, 1949) an attempt to control *Culicoides impunctatus* Goetghebuer in 1948 by means of the application of DDT residual sprays to vegetation was described. It was shown that the treatment had no discernible effect upon the midge population so that it was permissible to regard all the observations as having been made on an untreated population and to make direct comparisons between catching stations irrespective of their positions relative to the treated area.

In 1948 observations were made at two sites, Bannachra and Wester Bannachra, which were three-quarters of a mile apart and 300 ft. above Mean Sea Level on the north side of Ben Bowie, Loch Lomond. At each site equal numbers of observations were made under both woodland and moorland conditions so that in the following analysis reference will be made to four areas :—

(i) Bannachra Woodland
(ii) Bannachra Moorland
(iii) Wester Bannachra Woodland
(iv) Wester Bannachra Moorland

Regular weekly observations on the *C. impunctatus* populations were made at 44 catching stations, which were arranged as shown in fig. 1. There were 11 catching stations in each area, which were numbered 1–11 and prefixed by the initials of the area to which they belonged, *i.e.*, BW, BM, WBW and WBM. The stations in each area were so arranged that four were placed at the corners of a square (40 yards × 40 yards) with a fifth in the centre, *i.e.*, 28 yards from each of the others. This square was surrounded by a semicircle of six stations which were 40 yards away. The catching stations at each site covered an area 200 yards × 120 yards (=4·96 acres).

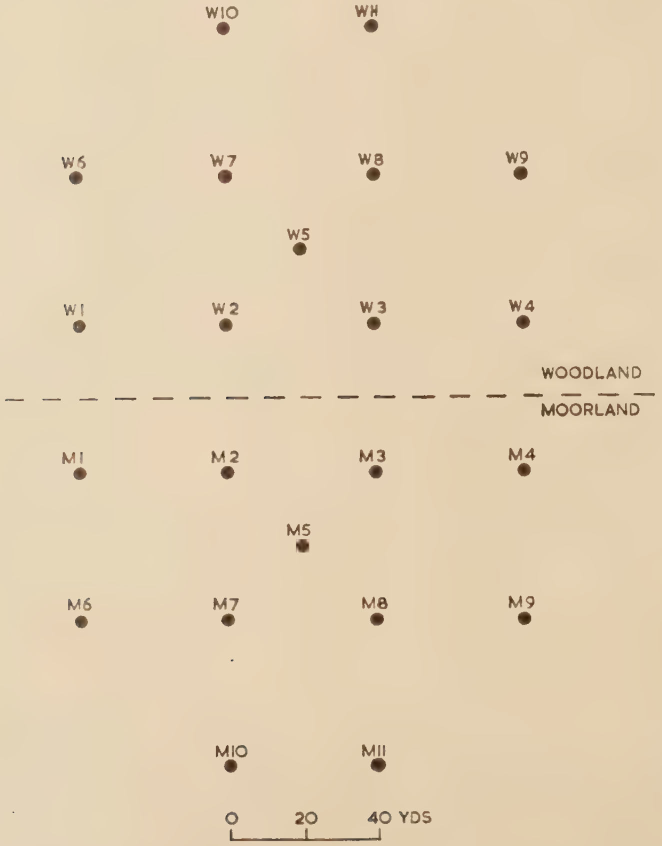

Fig. 1.—Arrangement of catching stations (closed circles) at Bannachra and Wester Bannachra. The Bannachra and Wester Bannachra stations have the prefix B and BW respectively when referred to in the text and tables.

Each catching station consisted of three metal cylinders (12 ins. high × 5 ins. diameter) placed vertically above one another on a wooden post so that their centres were 2 ft., 6 ft. and 10 ft. above the ground. To trap the midges each cylinder was covered with a sheet of celluloid (18 ins. × 12 ins.) treated with "Stopmoth"* a proprietary tree banding grease. The celluloid sheets were changed weekly and the ensnared midges removed, rendered grease free and preserved in 70 per cent. alcohol for later identification. This is substantially the same technique as that described by Broadbent and others (1948) with the exception that for *Culicoides* the cylinders were painted black instead of white because Hill (1947) had found dark colours to be attractive to female *Culicoides*.

Observations were begun in 1948 in the second week of May and continued until the end of the second week of September at Wester Bannachra (18 weeks) but on account of the illness of my assistant the observations at Bannachra were discontinued after 12 weeks at the end of the first week of August. However, this did not cause much loss of information since the catches during weeks one and two and 15–18 were insignificant.

Although all the traps were changed weekly it was not possible, on account of the pressure of other work, to remove the catches from all of the traps in the first and second weeks of June, 1948 (weeks four and five). At Wester Bannachra woods and Bannachra moorland 6 out of 11 and 7 out of 11 of the catching stations were examined, respectively, in the fourth and fifth weeks, whilst at Bannachra woods and Wester Bannachra moorland all the stations were examined in the fourth week and 6 out of 11 in the fifth week.

In the second week of June (fifth week) at Wester Bannachra and the fourth week of June (seventh week) at Bannachra the traps were exposed for nine days. The catches made in these weeks have been adjusted in the following manner.

$$a = b - \tfrac{1}{8}\,(b + c)$$

where a=adjusted catch of male or female *C. impunctatus* in weeks five or seven.

b=actual catch of male or female *C. impunctatus* in week five or seven.

c=actual catch of male or female *C. impunctatus* in the week following catch b, *i.e.*, week six or eight.

In 1948 the three traps at one station in each area were always orientated in the same manner. The exposed surfaces of the covering celluloids were divided into four equal vertical strips and placed on the cylinders in such a way that the centres of the strips faced N., S., E. and W. For convenience these traps will be referred to as directional traps, and they were set up at stations WBW1, WBM11, BW10 and BM10.

In 1949 observations were made only in Bannachra Woodland and at two stations (BM1 and BM2) on Bannachra Moorland. Trapping was carried out from the start of May until the end of August (17 weeks). On account of the small percentage (about 10 per cent.) caught on the 2 ft. traps in this area in 1948 only the 6 ft. and 10 ft. traps were operated in 1949.

(a) *Topography of Site.*

At Bannachra the experimental area was sited with its long axis running down the slope of the hill, the moorland being higher than the woodland (Pl. X, fig. 1 and text-fig. 2). To the west of the site there is a dense conifer plantation about twenty feet high which acts as a windbreak to the prevailing SW. wind. On the western side of the site the seepage down the moorland is held back by a dyke on the southern boundary of the woodland. This water flows at its eastern end into a stony gully unsuitable for midge breeding but at the westerly end it flows through gaps in the dyke and passes under the trees where it supports a luxuriant ground flora of *Sphagnum* spp. and *Juncus articulatus*.* Further into the woodland the water flows in a broad shallow depression along the western edge of the site, where *Luzula sylvatica* replaces *Juncus articulatus*. The dominant flora of the moorland is bracken (*Pteridium aquilinum*) interspersed with isolated trees and large clumps of fern predominantly the male shield fern, *Aspidium filix-mas*. The central part of the woodland site is composed of young birch saplings about 15 ft. high and supporting a very sparse ground flora. Surrounding the birch wood there is an older oak wood of more mature trees, about 40 ft. high bearing a dense ground flora of *Sphagnum* spp. and *Luzula sylvatica*.

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*Supplied by Joseph Bentley Ltd., Barrow-on-Humber, Lincolnshire.

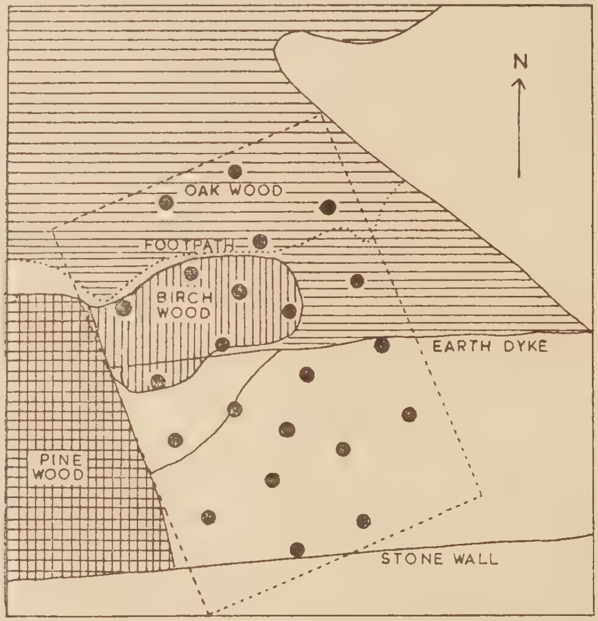

Fig. 2.—Diagrammatic representation of Bannachra site Plate 1a. Closed circles indicate catching stations and dotted line the boundary of the experimental area.

At Wester Bannachra the long axis of the site more or less follows a contour and as seen in Pl. X, fig. 2, and text-fig. 3, the moorland is almost devoid of trees. The flora of the central part of the moorland is composed of coarse grasses in which *Molinia caerulea*, the blue moor grass, *Deschampsia flexuosa* and *Anthoxanthum odoratum* abound. To the south of the site the seepage water is collected in a shallow swamp where *J. articulatus* dominates and then empties into a deep gully which flanks the western boundary of the site. To the north of the area there are swampy conditions around the broken dyke. The woodland at Wester Bannachra is composed solely of young birch saplings which are very dense on the moorland side with the exception of an open clearing in the centre (Pl. XI, fig. 3) but to the south the trees are more openly spaced and the intervening ground is colonised by bracken. In this area there are swampy conditions around the periphery of the woodland and at the moorland end of the open clearings.

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* In this paper *J. articulatus* is used as a collective term to include all large members of the Septati (*J. subnodulosus* (=*J. obtusiflorus*), *J. articulatus*, *J. acutiflorus* (=*J. sylvaticus*) and the hybrid between the last two species and another form " large 80 " of indeterminate status (Timm & Clapham 1940). In view of its geographical distribution and ecological requirements it is unlikely that *J. subnodulosus* occurs at Bannachra (Richards & Clapham 1941).

(b) *Specific status of* C. impunctatus.

In a recent paper (Kettle, 1950) there is a discussion on the possibility that *C. impunctatus* might consist of two biological races and it is concluded that there is sufficient evidence to justify the acceptance of this view at least as a working hypothesis. Therefore in the present paper the field results, obtained with *C. impunctatus*, have been analysed separately in the light of these two possibilities. In the first analysis it is assumed that *C. impunctatus* is a single specific unit and in the second that it is composed of two biological races.

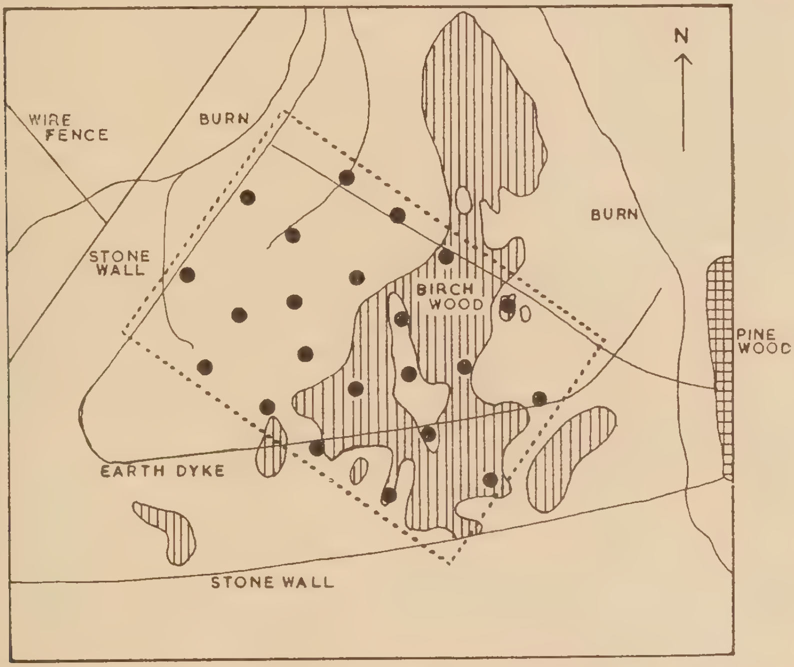

Fig. 3.—Diagrammatic representation of Wester Bannachra site Plate 1b. Closed circles and dotted line represent catching stations and the boundary of the experimental area respectively.

The races differ mainly in their seasonal distributions. One race appears in the spring and reaches a maximum at the start of June whilst the other appears about 6–8 weeks later and reaches its maximum towards the end of July. As a result the seasonal distribution of *C. impunctatus* taken as a whole, is bimodal with peaks in the first week of June and at the end of July. Between these maxima the *C. impunctatus* population falls to a minimum, at which time it will consist of a mixture of the two races. However, since the populations of both races rise and fall with great rapidity, the catches in the week before the minimum will be composed almost entirely of the spring race and those in the week after the minimum will be an almost pure sample of the summer race. Therefore when the results are analysed separately for each race, those for the weeks before the minimum are referred to the spring race, those for the weeks after the minimum to the summer race and the results in the week of the minimum are omitted.

In 1948 there was no difficulty in deciding the position of the minimum which occurred for both sexes in the third week of June in Wester Bannachra woods and in the fourth week of June in the other three areas except for the males on Bannachra moorland where the minimum occurred in the first week of July. In 1949 observations were restricted to Bannachra woodland but as a result of an unusually long dry spell of weather the summer race was considerably reduced and there are two possible positions for the minimum (for details see Kettle 1950). They are the fourth week in June and the third week in July but the first is probably the more accurate. However the summer race was so reduced that the catches were too small for analysis and hence the 1949 results refer exclusively to the spring race.

Horizontal Distribution of *C. impunctatus.*

(a) *Method of analysis.*

At first the actual weekly catches of male or female *C. impunctatus* at each station were tabulated separately for the four areas but it was not easy to make comparisons between the stations owing to the great fluctuations in the size of the *C. impunctatus* population during the season (Tables I–VIII).* The analysis of the seasonal distribution has been dealt with elsewhere (Kettle, 1950) so that here interest is centred upon the horizontal distribution. The effect of the seasonal changes can be eliminated by recording the catch of *C. impunctatus* at each catching station as a percentage of the total catch of *C. impunctatus* in the area. One result of making this adjustment is that the observations of each week have equal value. This is of great importance when it is desired to know whether the horizontal distribution of *C. impunctatus* has a particular arrangement which is constant from week to week.

When the weekly catches were converted to percentages the mean weekly percentage catch was calculated for each station and it became clear that there were marked differences between the percentage catches at different stations. However, the precise evaluation of these differences requires an estimate of the error of the means. The best estimate of this is obtained through the analysis of variance but this method can only be applied when it is reasonable to assume that the error of the means is the same for each station. A glance at the actual figures suggests that the error of the means is dependent upon the size of the mean, that is, the larger the mean the larger the error. Therefore the analysis of variance cannot be applied directly to the data. This difficulty can be overcome by submitting the weekly percentage catches to a square root transformation and this has been adopted here.

In the tables that follow the means of the transformed percentage values are given below the mean weekly percentage catches and these should be used in tests of significance in conjunction with $t_{0\cdot05}\, s_{\bar{d}}$ which is the value of the difference between the means for the 5 per cent. level of significance. That is, in a series of tests made on samples taken at random from the population, once in every twenty tests a difference as large as or larger than $t_{0\cdot05}\, s_{\bar{d}}$ will be obtained. This fact needs to be borne in mind when considering the results presented here, because often twenty or more comparisons are made and therefore it should be expected that the significance of one of them would exceed the 5 per cent. level merely as a result of random sampling,

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* The 25 Tables in this paper have been abbreviated from 41 Tables giving the full data. A complete set of the Tables has been deposited in the Archives of the British Museum (Natural History), Cromwell Road, London, S.W.7, and is available to interested workers.

TABLE I.

Weekly percentage distribution of male *C. impunctatus* in Bannachra woods in 1948. Values above upper line and those below lower line refer to spring and summer races respectively. $t_{0\cdot05}s_{\bar{d}}$=95 per cent. fiducial interval for differences between station or race means. F=variance ratio of between station variance. *Catch in 9 days and calculated catch in 7 days.

Week No.	B.W.1	B.W.2	B.W.3	B.W.4	B.W.5	B.W.6	B.W.7	B.W.8	B.W.9	B.W.10	B.W.11	No. of Males captured
3	11·6	38·7	22·2	4·8	13·2	1·1	2·1	2·1	2·6	1·1	0·5	189
4	8·7	19·4	4·8	12·7	16·6	6·9	8·6	13·2	4·9	3·1	1·1	652
6	10·1	46·8	15·3	1·5	11·9	2·8	8·2	1·5	0·9	0·6	0·3	327
7	8·9	34·8	12·5	8·9	9·4	12·0	4·7	2·6	1·6	3·6	1·0	192 (148)*
8	11·7	39·5	4·9	7·4	7·4	6·2	11·1	4·9	1·9	2·5	2·5	162
9	11·4	44·3	11·4	3·8	10·9	3·4	8·4	3·4	1·7	1·3	0·0	238
10	13·4	44·4	6·6	2·7	9·7	6·8	7·4	4·7	2·1	1·8	0·4	487
11	12·8	42·8	8·1	4·7	10·6	4·1	6·5	3·9	3·7	2·2	0·6	508
12	11·2	52·0	4·9	1·2	12·0	4·1	7·9	5·1	1·2	0·4	0·0	509
Station mean ...	11·1	40·3	10·1	5·3	11·3	5·3	7·2	4·6	2·3	1·8	0·7	3,263
Transformed station mean ...	3·32	6·30	3·06	2·17	3·34	2·20	2·64	2·04	1·46	1·29	0·70	$t_{0\cdot05}s_{\bar{d}}$ 0·58
Transformed mean of spring race	3·18	5·81	3·60	2·32	3·72	1·78	2·42	2·10	1·59	1·19	0·77	0·90
Transformed mean of summer race	3·48	6·67	2·64	1·92	3·17	2·20	2·86	2·09	1·43	1·23	0·60	

F=52·7 ($P_{0\cdot001}$, F=3·41)

TABLE II.

Weekly percentage distribution of *C. impunctatus* females in Bannachra woods 1948. For other details see Table I.

Week No.	B.W.1	B.W.2	B.W.3	B.W.4	B.W.5	B.W.6	B.W.7	B.W.8	B.W.9	B.W.10	B.W.11	No. of Females captured
3	13·6	37·5	13·6	3·4	12·5	0·0	12·5	2·3	1·2	3·4	0·0	88
4	11·3	17·1	4·7	9·4	16·8	8·1	6·6	14·9	5·3	3·7	2·1	381
6	11·1	34·6	11·5	4·7	14·1	5·6	6·0	3·4	4·2	2·6	2·2	234
7	16·2	29·3	8·9	10·6	9·7	9·7	6·5	1·7	3·3	3·3	0·8	123 (84)*
8	12·6	30·5	3·2	7·4	12·6	5·8	12·1	9·4	1·6	0·6	4·2	190
9	15·2	32·5	8·9	3·6	10·8	7·7	7·3	2·4	6·0	3·2	2·4	249
10	12·3	34·5	9·6	3·3	12·1	10·3	7·5	3·3	3·8	2·3	1·0	397
11	9·3	37·7	9·0	4·4	10·7	4·6	9·1	7·5	4·8	2·3	0·5	611
12	9·9	48·5	4·4	1·8	18·4	2·9	6·1	4·9	1·1	0·9	1·1	344
Station mean ...	12·4	33·6	8·2	5·4	13·1	6·1	8·2	5·5	3·7	2·5	1·6	2,617
Transformed station mean ...	3·51	5·76	2·80	2·24	3·60	2·28	2·83	2·21	1·80	1·51	1·13	$t_{0·05}s_{\bar{d}}$ 0·59
Transformed mean of spring race	3·46	5·38	3·09	2·36	3·80	1·74	2·85	2·41	1·81	1·85	0·97	0·91
Transformed mean of summer race	3·44	6·04	2·59	1·97	3·58	2·45	2·74	2·28	1·78	1·29	1·27	

$F=37·2 \quad (P_{0·001},\ F=3·51)$

TABLE III.

Weekly percentage distribution of *C. impunctatus* males on Bannachra moorland 1948. For other details see Table I.

Week No.	B.M.1	B.M.2	B.M.3	B.M.4	B.M.5	B.M.6	B.M.7	B.M.8	B.M.9	B.M.10	B.M.11	No. of Males
3	4·2	3·0	3·0	33·5	7·8	12·5	7·8	10·8	8·4	2·4	6·6	167
6	4·1	2·7	2·5	11·7	18·0	20·9	18·5	9·0	6·9	3·0	2·7	367
7	4·0	1·8	3·2	4·6	4·9	34·4	24·9	8·4	4·0	6·3	3·5	346 (272)*
8	0·8	0·4	1·2	4·9	6·6	50·6	13·5	6·1	4·5	3·7	7·7	245
9	7·0	0·2	3·6	4·9	13·3	31·9	14·7	10·7	2·4	3·1	8·2	414
10	4·6	2·2	2·5	4·8	6·4	30·2	17·7	8·6	3·7	11·3	8·0	861
11	8·1	4·3	3·7	3·7	8·0	30·2	11·8	17·7	4·8	5·2	2·5	1,124
12	7·8	3·0	4·3	5·8	14·3	16·3	23·1	14·0	3·6	4·2	3·6	307
Station mean ...	5·1	2·2	3·0	9·2	9·9	28·4	16·5	10·7	4·8	4·9	5·3	3,831
Transformed station mean ...	2·18	1·39	1·70	2·79	3·08	5·23	4·00	3·22	2·15	2·15	2·26	$t_{0·05}s_{\bar{d}}$ 0·73
Transformed mean of spring race	2·03	1·56	1·70	3·78	3·08	4·66	4·03	3·01	2·51	1·93	2·02	1·12
Transformed mean of summer race	2·60	1·45	1·87	2·18	3·20	5·18	4·07	3·54	1·89	2·38	2·29	

F = 18·2 ($P_{0·001}$, F = 3·59)

TABLE IV.

Weekly percentage distribution of *C. impunctatus* females on Bannachra moorland 1948. For other details see Table I.

Week No.	B.M.1	B.M.2	B.M.3	B.M.4	B.M.5	B.M.6	B.M.7	B.M.8	B.M.9	B.M.10	B.M.11	No. of Females
3	5·9	3·9	2·0	27·4	10·8	10·8	11·8	6·8	10·8	2·9	6·9	102
6	9·1	2·0	4·6	9·6	13·2	20·2	12·1	11·1	6·5	7·0	4·6	198
7	1·4	2·7	2·2	8·7	12·8	23·3	19·7	11·4	7·7	4·6	5·5	219 (166)*
8	1·9	3·9	2·9	6·9	7·8	44·9	12·7	3·4	3·9	2·9	8·8	205
9	7·8	4·2	8·7	5·6	10·3	19·8	12·5	13·9	4·4	3·3	9·5	359
10	5·1	3·4	3·5	5·7	8·5	22·1	15·7	14·1	4·1	8·2	9·6	728
11	8·1	4·3	5·0	2·6	9·4	28·4	10·7	17·6	4·6	5·6	3·7	1,054
12	10·8	6·4	6·9	9·4	12·3	13·8	11·3	11·8	6·4	5·0	5·9	203
Station mean ...	6·3	3·8	4·5	9·5	10·6	22·9	13·3	11·3	6·1	4·9	6·8	3,068
Transformed station mean ...	2·40	1·94	2·05	2·91	3·25	4·69	3·63	3·29	2·43	2·19	2·57	$t_{0\cdot05}s_{\bar{d}}$ 0·64
Transformed mean of spring race	2·73	1·69	1·77	4·17	3·46	3·89	3·46	2·97	2·93	2·19	2·38	1·08
Transformed mean of summer race	2·52	2·10	2·28	2·40	3·10	5·04	3·54	3·39	2·16	2·20	2·70	

$F=12\cdot9$ $(P_{0\cdot001}, F=3\cdot60)$

and not necessarily because it represents a real difference. Therefore tests of significance which do not exceed the 5 per cent. level can be regarded as statistically insignificant but the significance of any particular test, in which the 5 per cent. level is exceeded, needs to be carefully considered.

An estimate of the differences between the stations is the variance ratio (F) which is the ratio of the variance between stations to the variance within stations. The value of F is inserted in all suitable tables.

(b) *Bannachra 1948.*

The weekly percentage catches of *C. impunctatus* at the stations in Bannachra woods are given in Tables I and II. The variance ratios show that there is no doubt whatever about the existence of real differences between the stations in this area (P♂♂<0·001 ; P♀♀<0·001). Station BW2 has a significantly higher catch than any other station, trapping 40·3 per cent. of the males and 33·6 per cent. of the females, whilst stations BW9, 10 and 11 record catches which are significantly smaller than

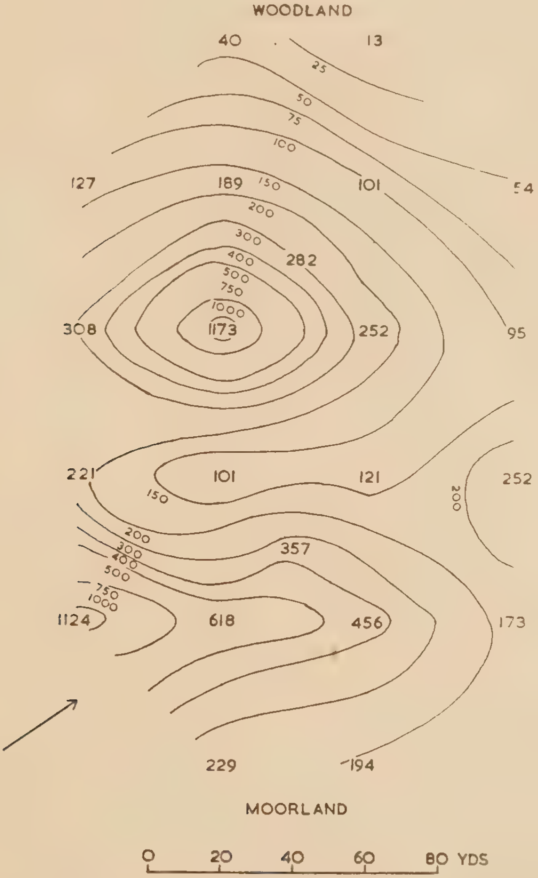
WOODLAND
40
13
25
50
75
100
127
189
150
101
54
200
300
282
400
500
750
1000
308
1173
252
95
221
101
121
200
252
150
200
300
400
500
357
750
1000
1124
618
456
173
229
194
MOORLAND
0
20
40
60
80 YDS

Fig. 4.—Distribution of male *C. impunctatus* at Bannachra. The large numbers refer to the actual catches at the various stations. The arrow gives the direction of the prevailing wind (SW.). The lines connect points of equal midge density, which is indicated by small numbers.

most of the others. In an attempt to understand the meaning of these marked consistent differences between the stations, it was decided to plot the *C. impunctatus* densities on a plan of the area. The results are shown in figs. 4 and 5, on which lines of equal midge density have been inserted on the assumption that the midge density falls off logarithmically between the stations. (The reason for this assumption will be given later p. 265). It will be noted that the midge density in the woodland falls off regularly with increasing distance from station BW2. The simplest interpretation of this result is that there is a relatively small concentrated breeding site near this station from which the midges are dispersing outwards in all directions. Support for this view is obtained by applying the X^2 test to the catches on the directional traps at station BW10. These indicate a significantly higher catch of males in the S. sector ($P<0{\cdot}05$), and a lower catch of females in the N. sector ($P<0{\cdot}01$), and, when the catches of the sexes are combined, a significantly lower catch in the N. sector ($P<0{\cdot}01$) and higher catch in the S. sector ($P<0{\cdot}05$). This would be expected since the S. sector faces and the N. sector is directed away from station BW2, *i.e.*, the breeding site. A more detailed analysis of this will be made later (p. 262).

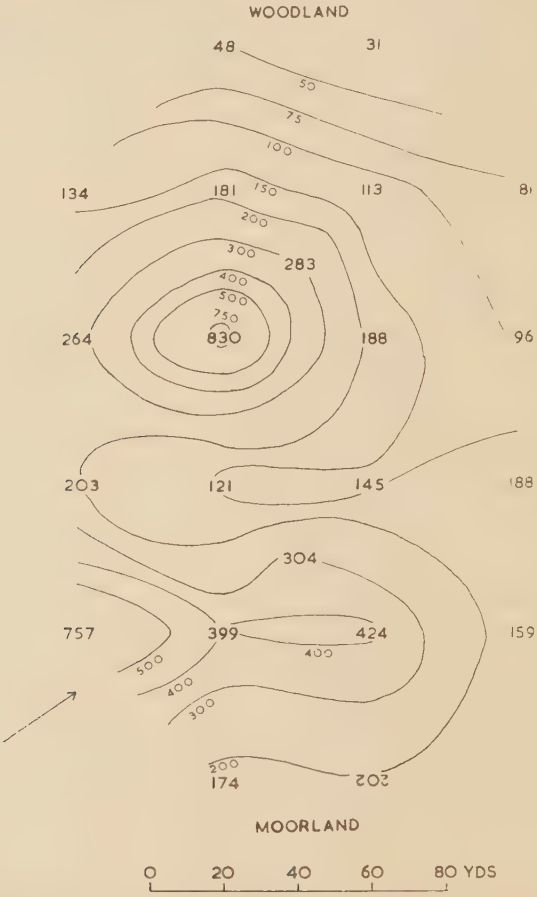
WOODLAND
48
31
50
75
100
134
181
150
113
81
200
300
283
400
500
750
264
830
188
96
203
121
145
188
304
757
399
424
159
400
500
400
300
200
174
202
MOORLAND
0
20
40
60
80 YDS

Fig. 5.—Distribution of female *C. impunctatus* at Bannachra. For other details see fig. 4.

The weekly percentage distribution of *C. impunctatus* on the moorland at Bannachra is given in Tables III and IV. The probabilities that the observed differences are due to experimental error are P♂♂<0·001 and P♀♀<0·001, indicating the existence of very real differences between the catches at the various stations. Thus BM6 has a significantly higher catch than any other station, trapping 28·4 per cent. of the males and 22·9 per cent. of the females, whilst BM2 and BM3 have significantly lower catches than most of the other stations. When the midge densities are mapped it is seen that they fall off regularly with increasing distance from BM6. There is a remote possibility that the stations furthest away from BM6 (BM4 and BM9) may be influenced by another breeding place (figs. 4 and 5). The lines of equal midge density are more elliptical than those in the woodland being elongated in the direction of the prevailing wind, which suggests that *C. impunctatus* is distributed partly by the wind. As in the woodland the distribution of the adults points to the existence of a relatively restricted breeding site near station BM6. Analysis of the catches at the directional station BM10 indicate a markedly higher catch (P♂♂<0·001; P♀♀<0·001), on the N. sector which faces BM6.

(c) *Wester Bannachra 1948.*

The weekly percentage catch at each station in Wester Bannachra woods is given in Tables V and VI. The observed differences between the stations are far too great to be the result of experimental error (P♂♂<0·001 ; P♀♀<0·001). In this area the catches at stations WBW3, WBW6 and WBW7 are significantly greater than those at most of the other stations whilst stations WBW4 and WBW9 record significantly lower catches than most of the others. When the *C. impunctatus* densities are plotted on a plan of the area (figs. 6 and 7) it is evident that there are two foci of high midge density centring around stations WBW3 and WBW6. Analysis of the catches on the directional traps at station WBW1 reveals that significantly more *C. impunctatus* of both sexes were obtained on the N. sector (P♂♂<0·001 ; P♀♀<0·001). This was an unexpected result since the two centres of high midge density lie to the SE. and SW. respectively of WBW1, and there appears to be no obvious explanation of this discrepancy.

The two areas of high density are separated by a belt of low density at stations WBW2 and WBW5. It is perhaps of interest that these latter stations are situated in a clearing in the wood (Pl. X, fig. 2 and Pl. XI, fig. 3). When the stations were erected nothing was known about the distribution of *C. impunctatus* in the area, indeed it was not even known whether this species occurred there but it was thought that the stations in the sheltered woodland clearing were in an ideal situation and yet as events proved they trapped relatively few midges.

On Wester Bannachra moorland the midges were more evenly distributed (Tables VII and VIII) but even so the probabilities that the observed differences were due to experimental error, were P♂♂<0·001 and P♀♀=0·01. In figs. 6 and 7 it will be seen that the *C. impunctatus* density is high on the periphery of the area and low in the centre, but there are no obvious centres of breeding, which is probably widespread around the periphery of this area. At the directional station WBM11 there was a significantly smaller catch of male* and female *C. impunctatus* on the E. sector (P♂♂<0·001 ; P♀♀<0·001), that is, on the sector facing the low midge density.

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*In the 11th week there was an unusually large number of male *C. impunctatus* captured on the 2 ft. trap and equally unusually they were nearly all on the N. sector. Thus 124/254 (49 per cent.) of the males caught in the 11th week were on the N. sector of the 2 feet trap compared with 86/540 (16 per cent.) in all the other weeks. Therefore the 11th week's results were regarded as "abnormal" and omitted from the calculation given above. If they are included the N. sector is found to have a high count and the E. sector a low count.

TABLE V.

Weekly pereentage distribution of *C. impunctatus* males in Wester Bannachra woods 1948. For other details see Table I.

Week No.	W.B.W.1	W.B.W.2	W.B.W.3	W.B.W.4	W.B.W.5	W.B.W.6	W.B.W.7	W.B.W.8	W.B.W.9	W.B.W.10	W.B.W.11	No. of Males
2	10·4	5·2	16·0	5·2	1·9	35·8	1·4	2·9	1·9	11·3	8·0	212
3	16·6	5·4	11·8	2·8	2·2	11·3	11·3	6·4	7·6	13·2	11·4	1,293
6	9·7	2·2	18·5	5·1	4·6	8·9	18·0	13·3	4·5	10·7	4·5	721
7	6·9	2·6	7·8	5·1	5·3	30·7	12·0	5·3	2·3	10·4	11·6	1,490
8	5·9	1·7	8·3	2·6	3·9	38·2	19·9	6·9	2·0	6·9	3·7	1,743
9	4·1	3·7	8·5	2·6	4·7	32·0	21·3	6·6	1·4	8·2	6·9	3,226
10	6·3	3·2	12·1	3·0	4·5	23·0	18·9	11·8	3·0	8·9	5·3	3,949
11	9·2	2·6	29·2	3·4	3·9	8·4	16·8	15·0	1·5	7·0	3·0	3,028
12	7·7	4·3	25·7	2·2	4·2	14·2	23·3	6·9	2·5	7·2	1·8	2,426
13	9·7	6·4	13·2	6·8	10·7	6·8	16·2	7·1	4·2	6·9	12·0	1,384
14	5·0	8·5	15·7	1·5	6·4	21·2	12·9	7·2	3·0	9·4	9·2	402
Station mean ...	8·3	4·2	15·2	3·7	4·8	21·0	15·6	8·1	3·1	9·2	7·0	19,874
Transformed station mean ...	2·80	1·97	3·80	1·87	2·13	4·36	3·83	2·78	1·70	2·99	2·56	$t_{0\cdot05}s_{\bar{x}}$ 0·66
Transformed mean of spring race	3·65	2·26	3·73	1·98	1·44	4·67	2·27	2·11	2·07	3·47	3·10	1·22
Transformed mean of summer race	2·56	1·95	3·75	1·79	2·30	4·47	4·16	2·83	1·55	2·84	2·48	

$F=14{\cdot}2$ $(P_{0\cdot001},\ F=3{\cdot}40)$

TABLE VI.

Weekly percentage distribution of *C. impunctatus* females in Wester Bannachra woods 1948. For other details see Table I.

Week No.	W.B.W.1	W.B.W.2	W.B.W.3	W.B.W.4	W.B.W.5	W.B.W.6	W.B.W.7	W.B.W.8	W.B.W.9	W.B.W.10	W.B.W.11	No. of Females
3	16·4	5·1	6·9	3·2	3·1	11·5	8·8	7·3	10·0	11·0	16·7	591
6	12·3	5·3	12·3	3·8	4·1	11·7	15·7	12·5	4·6	10·7	7·0	439
7	7·1	4·7	4·8	4·1	9·3	28·0	11·3	5·1	2·3	10·5	12·8	833
8	5·3	3·2	9·3	2·3	4·9	33·1	20·0	7·3	1·9	5·8	6·9	1,236
9	4·2	4·4	7·6	1·9	7·7	30·2	22·7	6·5	0·8	7·1	6·9	2,043
10	7·0	4·7	8·5	2·3	7·0	23·8	16·2	11·8	4·1	9·0	5·6	2,561
11	11·7	5·8	22·0	2·0	6·4	10·4	16·2	14·9	1·0	6·0	3·6	2,239
12	7·7	6·3	22·5	2·0	5·1	14·2	24·2	7·2	1·0	7·4	2·4	1,330
13	7·5	11·8	13·6	4·5	12·2	7·6	16·2	6·0	4·5	7·1	9·0	985
14	7·9	10·0	14·8	2·5	9·0	13·0	15·1	7·2	2·9	7·9	9·7	275
Station mean ...	8·7	6·1	12·2	2·9	6·9	18·4	16·6	8·6	3·3	8·2	8·1	12,535
Transformed station mean ...	2·90	2·43	3·40	1·68	2·57	4·16	4·04	2·89	1·69	2·85	2·75	$t_{0\cdot05}s_{\bar{d}}$ 0·59
Transformed mean of spring race	4·05	2·25	2·64	1·79	1·73	3·39	2·97	2·70	3·16	3·32	4·09	not calculated
Transformed mean of summer race	2·68	2·47	3·48	1·62	2·74	4·34	4·18	2·83	1·45	2·74	2·60	

$F = 14\cdot6$ ($P_{0\cdot001}$, $F = 3\cdot40$)

(d) *Interpretation of 1948 horizontal distribution.*

It has been shown that *C. impunctatus* adults are not uniformly distributed over the two experimental sites but occur in large numbers at a few catching stations, from which the midge density falls off quite regularly with increasing distance. Such a distribution would be obtained if *C. impunctatus* bred in small restricted sites from which the adults dispersed outwards into the surrounding country. If this were true then the high densities would indicate the proximity of breeding places. A search was made for the breeding site in Bannachra woods and it was located near to its expected position (this will be dealt with in more detail on p. 262). On account of the time-consuming nature of larval sampling it was not possible to look for the breeding sites in the other areas. Indirect support for this interpretation is obtained from the four directional stations. The results at three of them (BW10, BM10 and WBM11) indicate either high counts on the sector facing the breeding place or low counts on the sector directed away from it.

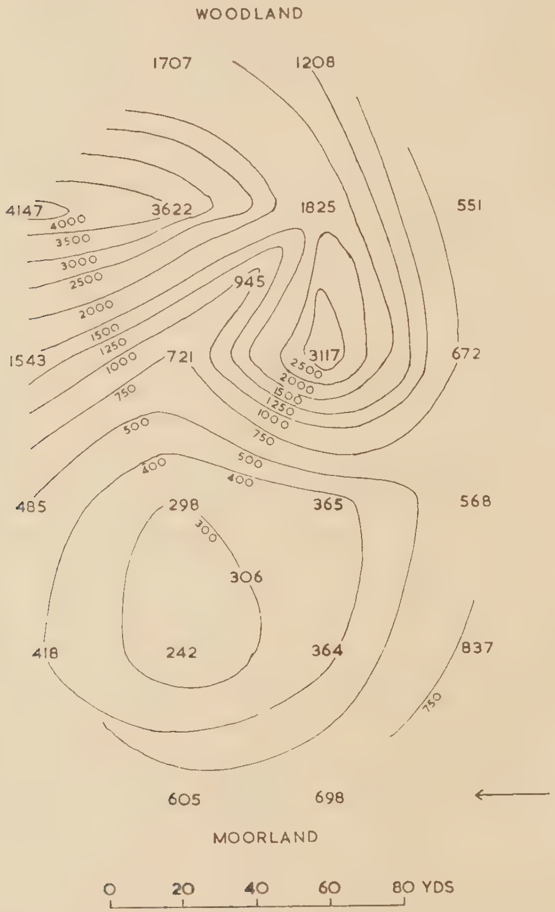
WOODLAND
1707
1208
4147
3622
1825
551
4000
3500
3000
2500
945
2000
1500
1250
1543
1000
721
3117
2500
672
2000
1500
1250
1000
750
750
500
500
400
400
485
298
300
365
568
306
418
242
364
837
750
605
698
MOORLAND
0
20
40
60
80 YDS

Fig. 6.—Distribution of male *C. impunctatus* at Wester Bannachra. For other details see fig. 4.

Two important conclusions can be drawn from this interpretation. Firstly, at Bannachra the *C. impunctatus* population over an area of at least 5 acres is coming from two small breeding places, and at Wester Bannachra the adult *C. impunctatus* population on the 5-acre experimental site is probably being maintained by two small breeding places in the woodland and one rather diffuse breeding zone on the moorland. Secondly, if this interpretation of the adult distribution is true then it is possible that *C. impunctatus* could be eliminated from large areas of countryside by treating the much smaller breeding areas with insecticides. To achieve this it is necessary to be able to recognise potential *C. impunctatus* breeding places on sight, since the search for larvae is too specialised and tedious for routine control measures. This aspect of the control of *C. impunctatus* is now being studied.

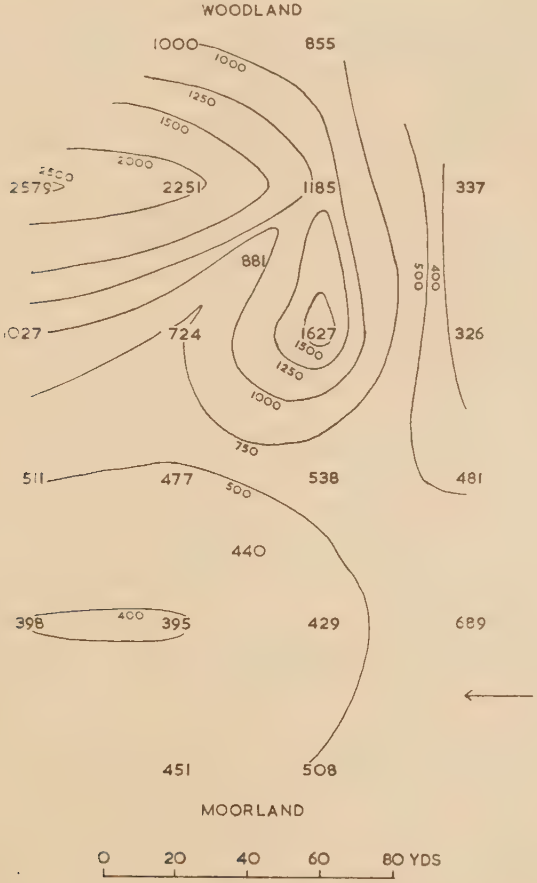
WOODLAND
1000
855
1000
1250
1500
2000
2500
2579
2251
1185
337
881
500
400
1027
724
1627
1500
1250
1000
326
750
511
477
500
538
481
440
398
400
395
429
689
451
508
MOORLAND
0
20
40
60
80 YDS

Fig. 7.—Distribution of female *C. impunctatus* at Wester Bannachra. For other details see fig. 4.

TABLE VII.

Weekly percentage distribution of male *C. impunctatus* on Wester Bannachra moorland 1948. For other details see Table I.

Week No.	W.B.M.1	W.B.M.2	W.B.M.3	W.B.M.4	W.B.M.5	W.B.M.6	W.B.M.7	W.B.M.8	W.B.M.9	W.B.M.10	W.B.M.11	No. of Males
3	9·4	7·7	7·0	25·6	4·6	11·6	8·3	8·7	5·4	7·4	4·3	300
4	7·1	10·5	6·5	19·8	6·8	10·0	4·2	3·8	8·5	12·8	10·0	918
6	14·6	4·3	7·7	8·8	7·3	8·1	8·4	5·8	8·9	16·9	9·2	260
7	11·2	6·5	9·2	10·6	7·3	9·8	4·6	4·0	12·5	15·1	9·2	152
8	5·4	4·6	5·8	5·8	11·3	13·3	5·0	9·2	11·7	21·7	6·2	240
9	10·5	5·3	8·4	7·2	7·3	5·8	3·3	6·3	16·2	18·0	11·7	695
10	10·3	5·5	9·0	15·0	6·9	8·0	6·1	6·0	12·3	12·1	8·8	872
11	5·0	5·0	6·0	9·1	5·9	5·5	2·6	9·0	15·8	11·0	25·1	1,011
12	9·2	4·8	5·2	8·2	4·4	11·2	3·9	5·2	27·6	8·5	11·8	596
13	11·9	7·2	6·2	15·2	3·1	7·2	4·5	5·1	20·2	6·1	13·3	720
14	8·2	8·3	7·1	8·2	4·1	7·1	5·6	8·6	21·0	9·0	12·8	267
Station mean ...	9·3	6·3	7·1	12·1	6·3	8·9	5·1	6·5	14·6	12·6	11·1	6,031
Transformed station mean ...	3·01	2·49	2·65	3·39	2·47	2·96	2·23	2·52	3·73	3·49	3·26	$t_{0\cdot05}s_{\bar{d}}$ 0·49
Transformed mean of spring race	3·18	2·69	2·65	4·17	2·49	3·13	2·61	2·43	2·74	3·47	2·77	0·79
Transformed mean of summer race	2·89	2·40	2·60	3·09	2·43	2·84	2·08	2·64	4·18	3·44	3·51	

$F=7\cdot96$ $(P_{0\cdot001}, F=3\cdot40)$

TABLE VIII.

Weekly percentage distribution of *C. impunctatus* females on Wester Bannachra moorland 1948. For other details see Table I.

Week No.	W.B.M.1	W.B.M.2	W.B.M.3	W.B.M.4	W.B.M.5	W.B.M.6	W.B.M.7	W.B.M.8	W.B.M.9	W.B.M.10	W.B.M.11	No. of Females
3	16·1	11·0	10·6	21·4	7·7	8·9	7·7	4·0	3·6	6·5	2·5	247
4	6·1	11·4	10·4	17·2	7·0	12·8	3·3	7·0	6·7	8·6	9·5	586
6	8·4	5·9	11·3	9·4	11·8	10·8	5·0	6·9	9·8	8·4	12·3	203
7	7·2	6·5	10·8	15·1	5·8	4·3	2·9	4·3	14·3	17·3	11·5	139
8	2·9	7·3	5·8	11·6	8·2	7·7	3·8	6·8	17·9	16·9	11·1	207
9	10·3	6·6	7·4	5·9	13·6	7·0	8·1	8·7	11·7	11·8	8·9	678
10	10·3	10·0	11·7	10·6	8·1	7·5	7·4	7·6	11·6	9·7	5·5	994
11	8·8	7·3	10·2	5·5	7·6	6·7	6·2	9·0	14·9	8·8	15·0	951
12	8·9	8·3	8·3	6·4	7·5	8·6	10·8	9·1	16·1	7·9	8·1	650
13	10·0	12·2	11·5	9·9	5·5	6·0	7·3	8·5	14·3	2·9	11·9	848
14	8·0	10·2	14·2	9·8	6·2	9·1	9·8	9·1	12·7	4·4	6·5	274
Station mean ...	8·8	8·8	10·2	11·2	8·1	8·1	6·6	7·4	12·1	9·4	9·3	5,777
Transformed station mean ...	2·92	2·94	3·18	3·27	2·82	2·83	2·51	2·69	3·43	2·98	2·89	$t_{0\cdot05}s_{\bar{d}}$ 0·46
Transformed mean of spring race	3·13	3·04	3·28	3·94	2·95	3·29	2·27	2·43	2·55	2·79	2·72	0·74
Transformed mean of summer race	2·87	2·96	3·11	2·89	2·82	2·74	2·74	2·89	3·76	2·89	3·06	

$F=2\cdot51\ (P_{0\cdot01},\ F=2\cdot53)$

(e) *Bannachra 1949.*

The weekly percentage catches of *C. impunctatus* at the stations in Bannachra woods are presented in Tables IX and X. As in 1948 the probabilities of the observed difference being accidental are *P*♂♂<0·001 ; *P*♀♀<0·001, and once again station BW2 has a significantly higher catch than any other station whilst as before stations BW9, BW10 and BW11 record very low catches. The transformed station means for 1948 and 1949 and the difference between the means are also given in Tables IX and X, and it will be noted that there is very close agreement between the means of the two years. The 5 per cent. level of significance is exceeded four times but since 22 comparisons are being made this level should be exceeded once without having any special meaning. Of these four significant differences three, BW♂♂3, BW♂♂7 and BW♂♂6, only just exceed the 5 per cent. level and are of doubtful importance but one BW♀♀7 has a probability of less than 1 per cent. Differences, which are the result of random error, are equally likely to occur in either sex at any station therefore the finding of significant differences in both sexes at one station (BW7) is evidence of a real difference in distribution between the two years, the most likely explanation of which, is that there has been an extension of the breeding place located near BW2 towards BW7.

(f) *Comparison of the horizontal distributions of the two races of* C. impunctatus.

In Tables I to VIII the transformed means of the two races are given separately for each station. (In 1949 the drought so reduced the summer race that the data were numerically insufficient and therefore Tables IX and X refer exclusively to the spring race.) For simplicity the difference between the means and their errors are presented in Table XI. Eighty-eight differences are recorded but only 77 can be tested because in Wester Bannachra woods no error of the means could be calculated for the females as there was only one observation on the spring race. There are a number of significant differences which compare as follows with those expected :—

P	<0·05	<0·02	<0·01	<0·001
Expected ...	3·85	1·54	0·77	0·08
Observed ...	9	7	7	1

Obviously the observed frequencies with which certain probabilities are found are much greater than anticipated. It is likely that sampling errors would affect only one sex at a station, whilst real difference between the races would be likely to affect both sexes at the same station. When three stations (WBM4, WBM9 and BM4) which have significant differences in both sexes, are excluded, the observed and expected frequencies become—

P	<0·05	<0·02	<0·01	<0·001
Expected ...	3·85	1·54	0·77	0·08
Observed ...	3	1	1	0

There is now close agreement between the observed and expected frequencies, therefore no importance should be attached to significant differences which are shown by only one sex at a station. This leaves three stations WBM4, WBM9 and BM4 to be considered. The probabilities, that the observed differences between the races at these stations were due to experimental error, are less than one chance in a hundred except for WBM♂♂9 which has a probability of less than one in a thousand. Therefore, there are real differences between the two races at these stations. Thus on Bannachra moorland BM4 records a much higher catch of the spring race than the summer race. At Wester Bannachra moorland there is a shift in the distribution of the two races. The spring race is more numerous at station WBM4 whilst the summer race is more abundant at station WBM9. Such a difference could be due either to the two races emerging from separate breeding places or to the effect of environmental factors, *e.g.*, wind direction. If the latter be true, it is

TABLE IX.

Weekly percentage distribution of male *C. impunctatus* in Bannachra woods 1949. $t_{0\cdot05}s_{\bar{d}}$ —95 per cent. fiducial interval ; F=between station variance ratio. x, xx=difference exceeds 5 per cent. or 2 per cent. level of significance.

Week No.	B.W.1	B.W.2	B.W.3	B.W.4	B.W.5	B.W.6	B.W.7	B.W.8	B.W.9	B.W.10	B.W.11	No. of Males
3	23·1	23·3	8·0	3·1	17·2	3·1	11·3	5·1	2·3	2·5	1·0	390
4	3·5	36·0	5·3	4·8	14·6	2·4	23·4	5·0	2·0	2·4	0·6	1,378
5	12·0	29·0	8·2	6·1	15·4	3·5	15·6	2·9	4·0	1·4	1·8	1,120
6	13·3	31·9	13·0	4·7	8·2	5·1	12·4	4·3	2·0	3·2	1·9	608
7	24·8	44·6	2·1	3·4	9·6	0·4	5·9	1·7	3·8	1·2	2·5	238
8	11·2	57·1	0·0	2·0	12·3	0·0	9·2	3·1	2·1	3·0	0·0	98
Station mean ...	14·7	37·0	6·1	4·0	12·9	2·4	13·0	3·7	2·7	2·3	1·3	3,832
Transformed station mean 1949	3·69	6·02	2·17	1·98	3·56	1·34	3·52	1·89	1·61	1·50	1·02	$t_{0\cdot05}s_{\bar{d}}$ 0·88
Transformed mean 1948 ...	3·32	6·30	3·06	2·17	3·34	2·20	2·64	2·04	1·46	1·29	0·70	0·58
Difference 1949–48	+0·37	−0·28	xx −0·89	−0·19	+0·22	x −0·86	xx +0·88	−0·15	+0·15	+0·21	+0·32	0·72

F—23·1 ($P_{0\cdot001}$; F=3·59)

TABLE X.

Weekly percentage distribution of female *C. impunctatus* in Bannachra woods 1949. xxx=difference exceeds 0·1 per cent. level of significance. For other details see Table IX.

Week No.	B.W.1	B.W.2	B.W.3	B.W.4	B.W.5	B.W.6	B.W.7	B.W.8	B.W.9	B.W.10	B.W.11	No. of Females
3	20·8	12·3	13·8	4·6	13·8	9·2	16·2	6·2	2·3	0·8	0·0	130
4	5·2	27·9	5·9	6·7	15·0	3·4	22·7	8·1	1·2	2·2	1·7	406
5	14·2	28·8	6·0	4·7	16·8	5·7	15·6	2·8	2·3	1·5	1·6	614
6	5·9	33·6	15·3	7·0	11·0	5·4	11·3	4·6	0·8	4·0	1·1	373
7	17·9	41·6	3·3	2·4	14·6	0·8	8·9	6·5	2·4	0·8	0·8	123
Station mean ...	12·8	28·8	8·9	5·1	14·2	4·9	14·9	5·6	1·8	1·9	1·0	1,646
Transformed station mean 1949	3·89	5·27	2·86	2·21	3·76	2·09	3·81	2·33	1·31	1·30	0·90	$t_{0\cdot05}S_{\bar{d}}$ 0·50
Transformed mean 1948 ...	3·51	5·76	2·80	2·24	3·60	2·28	2·83	2·21	1·80	1·51	1·13	0·59
Difference 1949–48	+0·38	−0·49	+0·06	−0·03	+0·16	−0·19	xxx +0·98	+0·12	−0·49	−0·21	−0·23	0·54

$F=60\cdot1$ ($P_{0\cdot001}$, $F=3\cdot93$)

difficult to understand why these factors should only affect stations WBM4 and WBM9 whilst the rest of the moorland stations remain unaffected. The more likely explanation is that the two races are breeding in closely related but not identical sites.

It can be concluded that although the distributions of the two races are substantially alike they are not identical. This is yet another pointer towards the existence of two races of *C. impunctatus*.

Flight Range of *C. impunctatus.*

(a) *Calculation of theoretical centre of breeding in Bannachra woods.*

Considerable curiosity was felt concerning the explanation of the regular distribution of *C. impunctatus* in the woods at Bannachra. In this area the lines of equal midge density are arranged concentrically around a centre near station BW2 (figs. 4 and 5). The simplest explanation of this distribution was that the adults were emerging from a relatively small breeding site near BW2 from which they dispersed outwards in all directions. It follows from this that equal midge densities should be the same distance from the centre of breeding. That is, positions of equal midge density can be regarded as occurring on the circumference of a circle whose centre is the breeding site. Now the centre of a circle lies on a perpendicular erected on and bisecting a line (chord) joining any two points on the circumference, so that the theoretical centre of breeding can be determined by erecting perpendiculars on lines joining stations of equal midge density. It is fortunate that in Bannachra woods the midge densities at many stations are more or less equal but to allow for the slight inequalities the perpendiculars were not erected at the centres of the chords but in positions determined from the following expression—

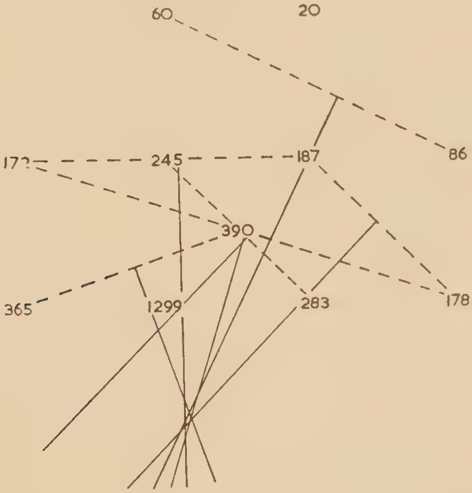

Fig. 8.—Calculation of theoretical centre of breeding. Continuous and broken lines represent perpendiculars and chords respectively. For other details see text.

$$a = \frac{d \times b}{b + c}$$

where a=distance of perpendicular from station y along chord
b=catch at station x
c= ,, ,, y
d=distance between x and y.

The result of this geometrical exercise is shown in fig. 8 and it will be noticed that five of the six perpendiculars converge on a small area. This diagram is based on the distribution of male *C. impunctatus* but similar conclusions were obtained with female *C. impunctatus* on its own and with both sexes combined.

(b) *Actual breeding site in Bannachra woods.*

Having calculated the theoretical centre of breeding it became important to determine it by direct observations on the distribution of *C. impunctatus* larvae in the soil. To do this the area around station BW2 was divided into squares (6 yds. × 6 yds.) and three soil samples were removed from each square. The samples were 3 ins. diameter and at least 2 ins. deep. Only the top 2 ins. were examined for larvae because it had been found earlier that 97 per cent. of the 3rd and 4th instar larvae occurred in that layer (Kettle, 1950). The technique of examining the samples was known to recover 1st instar larvae and it is believed that the recovery of 3rd and 4th instars was complete. Larval sampling can only be carried out when the larvae are in the 3rd or 4th instar since the 2nd instar cannot be identified with certainty. Therefore sampling was commenced on 25th February, 1949, and continued until 9th June, when the adult catches indicated that a considerable proportion of the larvae had pupated. The result of sampling around station BW2 was very disappointing. A few larvae were found on the moorland side of the station and odd ones on the woodland side but there was no sign of the expected concentrated breeding place (fig. 9).

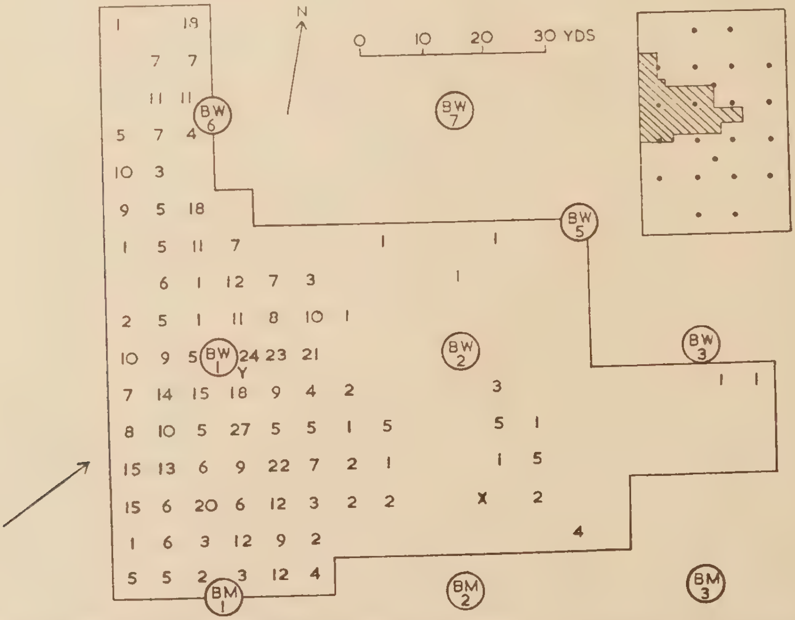

Fig. 9.—Results of larval sampling at Bannachra woods. The continuous line indicates the boundary of the sampled area. The figures refer to the number of *C. impunctatus* larvae, except nil, recovered from three samples in each square 6 yards by 6 yards. The arrow gives the direction of the prevailing wind. X and Y represent the theoretical and actual centres of breeding respectively. Inset the position of the sampled area (hatched) at Bannachra.

The search for larvae was extended therefore on all sides except the lower woodland side where the dry soil looked unsuitable. Quite soon large numbers of larvae were recovered around station BW1 and a dense breeding site was revealed.

Unfortunately the time factor prevented the exact limits of the site being found. However, judging from the nature of the ground and its flora, it is believed that very little of the breeding area was not sampled. Thus the larvae appeared to be associated with boggy ground bearing *Sphagnum* sp. and *Juncus articulatus*, whilst the unsampled ground east of BW6 including BW7 and BW5 and north of BW3 was raised dry ground supporting bracken in the small open clearing and almost devoid of a ground flora under the birch saplings. The western edge of the sampled area adjoins the dense pine wood seen in Plate X, and there is a very sudden and marked change in the nature of the terrain upon entering the wood. To the south of the area the apparently suitable larval conditions continue and it is likely that here the breeding place might extend further. Apart from this possible southerly extension it is felt that the limits of the breeding site are recorded in fig. 9.

The position of this breeding site was most puzzling. In the summer of 1948 the adults were most abundant at station BW2 and yet in the spring of 1949 the larvae were concentrated around station BW1. It was thought that there had been a shift in the breeding place and it was anticipated that in 1949 the adult population would also be greatest at BW1. However, it has already been demonstrated (p. 258) that apart from an increase in density at BW7 the adult distribution was the same in 1948 and 1949.

(c) *Explanation of low catch at BW1.*

To find out whether the unexpectedly low count in 1949 might be due to the adults flying low over the breeding place, the 2 ft. trap was exposed at BW1 from the second week in June and it caught 74/289 (=26 per cent.) of the total compared with 49/286 (=17 per cent.) in 1948. Therefore the low population density at BW1 was not due to any large increase in the number of adults flying below the 6 ft. trap.

It was noticed that the *C. impunctatus* adults were not uniformly distributed over the surface of the traps at BW1 but were congregated on the portion facing west. To obtain accurate data on this the 6 ft. trap was converted into a directional one at stations BW1, BW2, BW5 and BW7, from the third week in June. Unfortunately an unusually long spell of hot dry weather caused a considerable reduction in the *C. impunctatus* population for the rest of the season and reduced the usefulness of this procedure. At station BW7 the catches on the four sectors were low and revealed no significant differences. At BW5 the X^2 test indicated that the probability that equal numbers of adults were caught in all four sectors was less than one in fifty and that this was due to the low catch on the sector facing east because when this was excluded P had a value between 0·3–0·5. At BW2 the E. sector caught significantly less males and females than the others (P♂♂$<0{\cdot}001$;

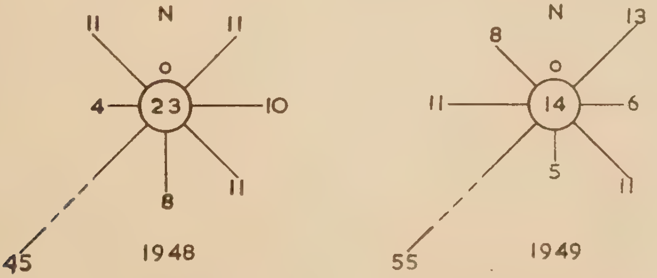

Fig. 10.—Direction and frequency of winds at Valvehouse meteorological station (3 miles from Bannachra) during May-August (123 days) in 1948 and 1949. The numbers refer to the number of days on which the wind blew from each direction. The figure in the circle records the number of calm days.

P♀♀$<0{\cdot}001$) and when both sexes were added together the W. sector had a significantly higher count ($P=0{\cdot}01$) than either the S. or N.

At BW1 the catch on the N. sector was significantly higher than on the others ($P<0{\cdot}001$) and that on the W. sector was higher than that of either the E. or S. sector ($P<0{\cdot}01$ nearly $0{\cdot}001$). This indicated that the midges were coming to BW1 from the N. and W., which is most astonishing considering that the greater part of the breeding site is situated to the S. and E. This result is due almost entirely to the catches in one week, the third week in June, and in this week BW2 also recorded significantly higher catches on its N. and W. sector ($P<0{\cdot}001$) yet over the whole season BW2 had a higher catch on its W. sector (see above). It is interesting to note that in the third week in June the direction of the wind was NE. on three days, NW. on two days, W. on one day and SE. on the other day.* Such a predominance of northerly winds is most unusual (see fig. 10). Over a period of four months May to August (123 days) on only 21 days (one in six) was the wind NW., N. or NE. and yet in the third week of June the wind was from this quarter on five days. It seems likely that the high catch on the N. sector of BW1 was directly attributable to this cause. When this week is omitted the data are inadequate for analysis. However, by comparison with BW2 and bearing in mind the earlier general observation that the adults were largely on the W. sector of BW1, it can be tentatively accepted that the *C. impunctatus* adults impinged on station BW1 from the west.

If the movement of *C. impunctatus* was at random, that is, that any individual in any position was likely to move in any direction, there would have been no difference between the catches in the four sectors. The existence of highly significant differences in these catches is proof that *C. impunctatus* adults did not move at random but tended to disperse in definite directions. Thus station BW2 records a significantly higher catch on its W. sector facing the breeding site and a lower catch on its E. sector and BW5 also catches less on its E. sector. These results might merely indicate that once an adult *C. impunctatus* leaves the breeding place it continues to fly in the same direction. Therefore stations BW2 and BW5 would only be affected by those individuals which fly E. This does not explain the anomaly of station BW1, which, being in the centre of breeding should receive adults from all sides, particularly from the south. Evidence has already been presented, which indicates that BW1 receives midges largely from the west. If that is true then it is at once clear that BW1 will catch relatively few midges because only a small fraction of the breeding place is to the west of this station and such an explanation will also account for the low catches at stations BW6 and BM1. Both stations were in the midst of the breeding area and yet trapped less midges than any other station except BW9, BW10 and BW11.

What is the reason for this eastward movement of the *C. impunctatus* population? It has already been noted that the lines of equal midge density on Bannachra moorland are elongated in the direction of the prevailing wind (SW.) and that in the third week of June the catches were highest on the N. and W. sectors of the directional traps as a consequence of the NE. and NW. winds. It is likely, therefore, that the eastward movement is brought about by the prevailing SW. winds, fig. 10.

(d) *Density coefficient of* C. impunctatus.

The density of *C. impunctatus* at a station will depend upon its distance from the breeding place providing that the station is to the east of the site. All stations except BW1 and BW6 are east of the breeding place. As a basis for calculation it will be assumed that stations BW1 and BW6 are only affected by that part of the breeding place which is to the west., *i.e.*, which lies between SW. and NW. The area enclosed by these two bearings is one-thirteenth of the total breeding area so that on the assumption that the larval density is approximately constant throughout the breeding place the catches at stations BW1 and BW6 need to be increased by ×13 before they can be compared with those of other stations. This correction has been applied in all the calculations which follow.

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* Through the courtesy of the Burgh Surveyor, Helensburgh, meteorological data were made available for Valvehouse meteorological station 3 miles from and at the same elevation (293 ft. above M.S.L.) as the Bannachra site

A centre of the breeding area has been calculated so that there are equal amounts of breeding N. and S. of an E.-W. line through this centre and equal amounts E. and W. of a N.-S. line. This centre (Y) is inserted in fig. 9 and is 45 yds. NW. of the theoretical centre (X).

Various formulae have been proposed to express the relationship between insect density and distance from the breeding place. Wadley and Wolfenbarger (1944) suggest the use of the formulae

$$y=a+b\log x \quad\ldots\ldots\ldots\ldots 1.$$

and $$y=a+b\log x+\frac{c}{x} \quad\ldots\ldots\ldots\ldots 2.$$

where $y=$ insect density at x
$a=$ " position on graph "
$\left.\begin{matrix} b \\ \text{and } c \end{matrix}\right\}=$ " are factors determining the slope of the curve as influenced by the logarithm or reciprocal of the distance."

Wolfenbarger (1946) in an exhaustive survey of the literature has applied these formulae to all available published data. Gregory and Read (1949) have used the expression

$$\log I=a+bx \quad\ldots\ldots\ldots\ldots 3.$$

to indicate the relationship between the number of infective punctures (I) on plants at a distance x from the source and a and b are constants. Since I will depend upon the insect density this can be regarded as an insect density/distance expression. This formula has been used here and was found to give a better fit to the data than formulae 1 and 2 above. It has been applied in the form

$$\log y=\log a+bx \quad\ldots\ldots\ldots\ldots 4.$$

where $y=$ *C. impunctatus* density at x
$a=$ *C. impunctatus* density at the centre of breeding
$b=$ regression coefficient ;
and $x=$ distance from breeding centre in yards.

The regression coefficient is always negative because the density (y) decreases as the distance from the breeding place (x) increases. The following expressions have been calculated.

C. impunctatus Males.

Population	Expression	Regression Coefficient
1948	log. $y=3{\cdot}669-0{\cdot}0165x$ .... (5)	0·0165 ±0·0028
1949	log. $y=3{\cdot}732-0{\cdot}0157x$ .... (6)	0·0157 ±0·0018
1948+1949	log. $y=4{\cdot}005-0{\cdot}0160x$ .... (7)	0·0160 ±0·0021
Spring race 1948 ...	log. $y=3{\cdot}084-0{\cdot}0144x$ .... (8)	0·0144 ±0·0029
Summer race 1948 ...	log. $y=3{\cdot}499-0{\cdot}0182x$ .... (9)	0·0182 ±0·0027

C. impunctatus Females.

Population	Expression	Regression Coefficient
1948	log. $y=3{\cdot}541-0{\cdot}0151x$ .... (10)	0·0151 ±0·0024
1949	log. $y=3{\cdot}466-0{\cdot}0162x$ .... (11)	0·0162 ±0·0019
1948+1949	log. $y=3{\cdot}798-0{\cdot}0154x$ .... (12)	0·0154 ±0·0021
Spring race 1948 ...	log. $y=2{\cdot}903-0{\cdot}0135x$ .... (13)	0·0135 ±0·0025
Summer race 1948 ...	log. $y=3{\cdot}381-0{\cdot}0156x$ .... (14)	0·0156 ±0·0023

The most important part of these expressions is the regression coefficient which represents the rate at which the midge density falls off with increasing distance from the breeding place. Although the regressions vary in magnitude from 0·0135±0·0025 to 0·0182±0·0027 none of the differences is significant. That is, the discrepancies between the regression coefficients of the same sex—firstly in 1948 and 1949, and secondly between the spring and summer races—is attributable to experimental error, as are also the observed differences between the regression of the two sexes. Therefore in fig. 11 only the combined results for 1948 and 1949 have been illustrated.

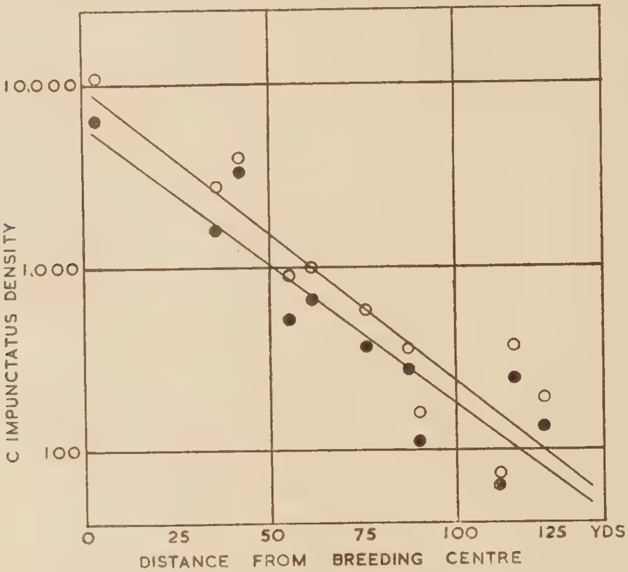

Fig. 11.—The effect of distance from the breeding place on the density of *C. impunctatus*. Closed circles indicate females and open circles indicate males. The upper (male) and lower (female) lines are the regression lines fitted to the observations. Ordinate= midge density on a logarithmic scale. Abscissa=distance from breeding centre in yards.

A useful derivative of the regression coefficient is its reciprocal, the density coefficient, which may be defined as the distance from the breeding place at which the density has fallen to one-tenth of its value at the centre. If the reciprocal of the regression coefficient $(1/b)$ is substituted for x in formula 4 then bx becomes 1 and $\log. y = \log. a - 1$. At the centre of breeding $x=0$ and $\log. y = \log. a$. Therefore as x increases from 0 to $1/b$ $\log. y$ decreases from $\log. a$ to $\log. a-1$, that is, y decreases from a to $1/10\ a$. The density coefficients for male and female *C. impunctatus* are 62·5 yds. and 64·9 yds. respectively. This estimate is most useful in the planning of control measures against *C. impunctatus* because the annoyance caused by this species is directly related to its density and at 64·9 yds. this is one-tenth of the density at the centre of breeding and at 129·8 yds. $(=2/b)$ it is 1/100th so that control measures should be adequate if all breeding places within 100 yds. are controlled providing that no very dense breeding site is left uncontrolled to the windward of the area to be protected.

(e) *Effect of dilution and mortality on density**

In this work the size of the *C. impunctatus* population has been estimated by trapping the adults on sticky traps. The number trapped in any given period will depend upon the activity of the midges. If the activity of an individual is doubled in all probability its chances of being trapped in any given period will also be doubled but the time it takes to pass a trap will be halved and therefore its likelihood of being captured remains unaltered. Hence the number of adults trapped at a station is a constant proportion of those which pass by and this proportion is independent of the speed at which they do so.

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* I am indebted to Dr. R. A. Robb of the Mathematics Department, Glasgow University, for assistance with this and the succeeding section.

The decrease in midge density with increasing distance from the breeding place is brought about by two separate processes. One, which can be termed the " dilution " effect, is the result of the midges being spread over an ever-increasing area and the other, the consequence of some adults going to earth or dying. This latter can be termed the " mortality " effect. It has been shown that at Bannachra *C. impunctatus* moves eastward from the breeding place and if every midge kept on the same bearing there would be no " dilution " effect because all the adults emerging from any one " spot " would take the same route so that over the whole season the same number of midges would pass over any other " spot " on the route ; that is, the density at these " spots " would be the same. However, the eastward movement is not so rigid. This is shown by the fact that at stations BW2 and BW5 there is no significant difference between the catches of male and female *C. impunctatus* on the N., S. and W. sectors except when the results for the sexes are pooled at BW2. Whereas if the midges were moving on a constant bearing one sector, or at the most two, would record higher catches and there would never be three sectors with equal catches. Also in 1948 BW10 recorded a higher catch on its S. sector, the one facing the breeding place. Therefore the movement of *C. impunctatus* shows wide variation about its general eastward direction so that " dilution " does play a part in reducing the density. The direction of this eastward movement can be represented as E±c°.

TABLE XI.

Difference between transformed means of the spring and summer races in 1948 : $t_{0\cdot05}s_{\bar{d}}$ =95 per cent. fiducial interval for difference between means. x, xx, xxx and xxxx indicate a difference which exceeds the 5 per cent., 2 per cent., 1 per cent. or 0·1 per cent. level of significance.

Station Number	Bannachra Woodland		Bannachra Moorland		Wester Bannachra Woodland		Wester Bannachra Moorland	
	Males	Females	Males	Females	Males	Females	Males	Females
1	−0·30	+0·02	−0·57	+0·21	+1·09	+1·37	+0·29	+0·26
2	−0·86	−0·66	+0·11	−0·51	+0·31	−0·22	+0·29	+0·08
3	+0·96	+0·50	−0·17	−0·51	−0·02	−0·84	+0·05	+0·17
4	+0·40	+0·39	xxx +1·60	xxx +1·77	+0·19	+0·17	xxx +1·08	xxx +1·05
5	+0·55	+0·22	−0·12	+0·36	−0·86	−1·01	+0·06	+0·13
6	−0·42	−0·71	−0·52	x −1·15	+0·20	−0·95	+0·29	+0·55
7	−0·44	+0·11	−0·04	−0·08	xxx −1·89	−1·21	+0·53	−0·47
8	+0·01	+0·13	−0·53	−0·42	−0·72	−0·13	−0·21	−0·46
9	+0·16	+0·03	+0·62	+0·77	+0·52	+1·17	xxxx −1·44	xxx −1·21
10	−0·04	+0·56	−0·45	−0·01	+0·63	+0·58	+0·03	−0·10
11	−0·41	−0·71	−0·27	−0·32	+0·62	+1·49	−0·74	−0·34
$t_{0\cdot05}s_{\bar{d}}$	0·90	0·91	1·12	1·08	1·22	Not calculable	0·79	0·74

TABLE XII.

The effect of " mortality " on the population size (Nx) at varying distances from the centre of breeding and the average distance flown by *C. impunctatus* males.

Distance from centre of breeding x	Density of male *C. impunctatus* y	Nx $=2\pi xyk\Delta x$	Interval	Average Distance Flown
Yards		$k\Delta x$	$k\Delta x$	Yards
10	6,998	439,698		
15	5,821	548,617		
20	4,842	608,465		
25	4,027	632,561		
30	3,350	631,461	1,100	27·5
35	2,786	612,674	18,787	32·5
40	2,317	582,326	30,348	37·5
45	1,928	545,168	37,158	42·5
50	1,603	503,598	41,570	47·5
55	1,334	460,998	42,600	52·5
60	1,109	418,084	43,914	57·5
65	922·6	376,797	40,287	62·5
70	767·4	337,520	39,277	67·5
75	638·3	300,792	36,728	72·5
80	530·9	266,860	33,932	77·5
85	441·6	235,846	31,014	82·5
90	367·3	207,703	28,143	87·5
95	305·5	182,354	25,349	92·5
100	254·1	159,656	22,698	97·5
105	211·3	139,402	20,254	102·5
110	175·8	121,504	17,898	107·5
115	146·2	105,639	15,865	112·5
120	121·6	91,684	13,955	117·5
125	101·2	79,482	12,202	122·5
130	84·1	68,694	10,788	127·5
135	70·0	59,376	9,318	132·5
140	58·2	51,195	8,181	137·5
145	48·4	44,095	7,100	142·5
150	40·3	37,981	6,114	147·5
155	33·5	32,625	5,356	152·5
160	27·9	28,048	4,577	157·5
165	23·2	24,052	3,996	162·5
170	19·3	20,615	3,437	167·5
175	16·0	17,592	3,023	172·5
180	13·3	15,041	2,551	177·5
185	11·1	12,902	2,139	182·5
190	9·2	10,983	1,919	187·5
195	7·7	9,434	1,549	192·5
200	6·4	8,042	1,392	197·5
210	4·4	5,805	2,237	205
220	3·0	4,146	1,659	215
230	2·1	3,041	1,105	225
240	1·5	2,261	780	235
250	1·0	1,570	691	245
			1,570	255

It is possible that the movement is not unidirectional but that the adults move outwards at first and then return to the breeding site. If there were no mortality this behaviour would merely double the catches at all stations and leave the regression coefficient unaltered. If, however, there is a steady mortality many more adults will turn back than will eventually arrive at the breeding site. Therefore, the result of this behaviour would be relatively to increase the adult density at stations away from the breeding site and thus reduce the size of the regression coefficient.

Now if x be the radius of one circle and $x+\Delta x$ the radius of another circle with the same centre then the area between the two circles is—

$$\begin{aligned}
&=\pi(x+\Delta x)^2-\pi x^2\\
&=\pi x^2+2\pi x\Delta x+\pi\Delta x^2-\pi x^2\\
&=2\pi x\Delta x+\pi\Delta x^2\\
&=2\pi x\Delta x \text{ (assuming } \Delta x \text{ is small)}
\end{aligned}$$

and the area enclosed by a sector whose angle is $2c°$ is

$$\frac{(2\pi x\Delta x)\ (2c°)}{360}$$

let $2c/360=k$

then the area $=k2\pi x\Delta x$

Since Δx is small the midge density between x and $x+\Delta x$ can be assumed to be constant and equal to y so that the number of midges (Nx) in the sector is

$$Nx=k2\pi x\Delta xy \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 15$$

The value of y for any given x can be obtained from expressions 7 and 12 (p. 265) and therefore Nx can be calculated for all values of x. This has been done for $x=5$ yds. to $x=200$ yds. at 5-yd. intervals and $x=200$ yds. to $x=250$ yds. at 10-yd. intervals (Tables XII and XIII). It is found that Nx increases rapidly at first to a maximum at 25 yds. for males and 30 yds. for females after which Nx decreases logarithmically. The initial increase is a mathematical consequence of reducing the breeding place to a theoretical point, the centre of breeding. It has no biological significance, and therefore the readings of Nx for $x<25$ yds. (males) and $x<30$ yds. (females) can be disregarded. The values of Nx are obtained in terms of two constants, $k\Delta x$, and are, therefore, relative and not absolute values.

Since Nx is the product of the density and area it is independent of the dilution effect so that the decrease in Nx as x increases is due probably to the mortality effect. There is another possible cause of a reduction in the size of Nx. If the directional impulse, whatever it may be, which begins the eastward movement were to become progressively weaker, as the distance from the breeding place increases, the dispersion will not always be on the bearing $E\pm c°$ where $c°$ is a constant but $E\pm c_1°$ where $c_1°$ increases with x. This would also produce a reduction in Nx but there is no means of assessing the importance of this factor. For the present this effect must be incorporated in the mortality effect.

If there were no mortality effect Nx would be constant and taking the maximum value of Nx, *i.e.*, $x=30$ for females and $x=25$ for males, as indicating the initial size of the population the expected density of *C. impunctatus* for all values of x can be calculated from formula 15 as follows—

$$Nx = k2\pi x\Delta xy \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 15$$

therefore for males

$$y\ ♂♂ = \frac{N_{25}}{k2\pi x\Delta x}$$

$$y\ ♂♂ = \frac{632{,}561\ k\Delta x}{k2\pi x\Delta x}$$

$$y\ ♂♂ = \frac{632{,}561}{2\pi x} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 16$$

and for females

$$y\ ♀♀ = \frac{N_{30}}{k2\pi x\Delta x}$$

$$y\ ♀♀ = \frac{408{,}659\ k\Delta x}{k2\pi x\Delta x}$$

$$y\ ♀♀ = \frac{408{,}659}{2\pi x} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 17$$

The values of y ♂♂ for $x=25$ yds. to $x=200$ yds. and y ♀♀ from $x=30$ yds. to $x=200$ yds. have been calculated at 5-yd. intervals and are graphically illustrated in fig. 12. These values (continuous lines) indicate the expected decrease in density with increasing distance as a result of dilution whereas the broken lines give the densities calculated from expressions 7 and 12. The difference between the two curves is an estimate of the mortality effect.

TABLE XIII.

The effect of " mortality " on the population size (Nx) at varying distances from the centre of breeding and the average distance flown by female *C. impunctatus*.

Distance from centre of breeding x	Density of female *C. impunctatus* y	Nx $=2\pi xyk\Delta x$	Interval	Average Distance Flown
Yards		$k\Delta x$	$k\Delta x$	Yards
10	4,406	276,828		
15	3,690	347,775		
20	3,090	388,302		
25	2,588	406,523		
30	2,168	408,659		
35	1,816	399,360	9,299	32·5
40	1,521	382,270	17,090	37·5
45	1,274	360,241	22,029	42·5
50	1,067	335,209	25,032	47·5
55	893·3	308,703	26,506	52·5
60	748·2	282,065	26,638	57·5
65	626·6	255,908	26,157	62·5
70	524·8	230,820	25,088	67·5
75	439·5	207,110	23,710	72·5
80	368·1	185,028	22,082	77·5
85	308·3	164,654	20,374	82·5
90	258·2	146,009	18,645	87·5
95	216·3	129,110	16,899	92·5
100	181·1	113,789	15,321	97·5
105	151·7	100,082	13,707	102·5
110	127·1	87,845	12,237	107·5
115	106·4	76,881	10,964	112·5
120	89·1	67,180	9,701	117·5
125	74·6	58,591	8,589	122·5
130	62·5	51,051	7,540	127·5
135	52·4	44,447	6,604	132·5
140	43·9	38,616	5,831	137·5
145	36·7	33,436	5,180	142·5
150	30·8	29,028	4,408	147·5
155	25·8	25,126	3,902	152·5
160	21·6	21,715	3,411	157·5
165	18·1	18,765	2,950	162·5
170	15·1	16,129	2,636	167·5
175	12·7	13,964	2,165	172·5
180	10·6	11,988	1,976	177·5
185	8·9	10,345	1,643	182·5
190	7·4	8,834	1,511	187·5
195	6·2	7,596	1,238	192·5
200	5·2	6,534	1,062	197·5
210	3·7	4,882	1,652	205
220	2·6	3,593	1,289	215
230	1·8	2,601	992	225
240	1·3	1,960	641	235
250	0·9	1,414	546	245
			1,414	255

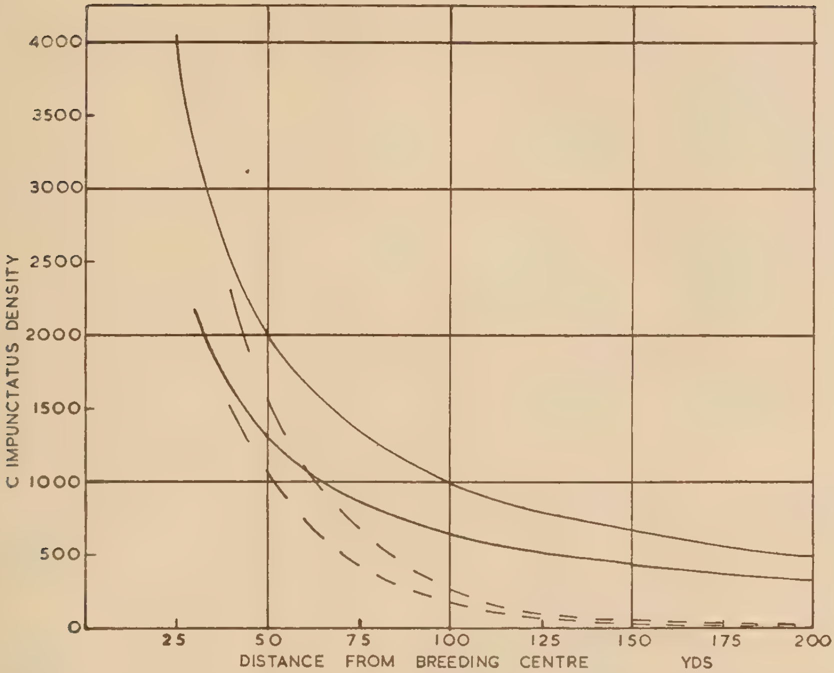

Fig. 12.—The effect of " mortality " on the density of *C. impunctatus* at varying distances from the centre of breeding. The continuous and broken lines indicate respectively the expected midge density if there were no mortality and the actual observed density. The upper line of each pair refers to the males and the lower to the females. Ordinate and abscissa represent respectively *C. impunctatus* density and distance from the breeding place in yards.

This effect can be isolated in the following way. Let the expected density be p and the observed density q at a distance x yards from the breeding site then the mortality will be $p-q$ and the percentage mortality—

$$\frac{p-q}{p} \cdot 100$$

This calculation has been carried out at 10-yd. intervals of x from $x=40$ yds. to $x=170$ yds. It was found that when the percentage mortality was plotted against the distance in yards an asymmetrical sigmoid curve was produced. This asymmetry disappeared when the logarithm of the distance was used instead of the distance. If the mortality is normally distributed with respect to the logarithm of the distance then there should be a linear relationship between the percentage mortality in terms of probits and the logarithm of the distance. This is found to be the case (fig. 13) and the mortality distributions can be expressed as follows in terms of the logarithm of the distance—

Males $1{\cdot}867 \pm 0{\cdot}197$
i.e., 73·6 yds. (46·8 yds. to 115·9 yds.)
Females $1{\cdot}885 \pm 0{\cdot}197$
i.e., 76·7 yds. (48·8 yds. to 120·8 yds.)

The means represent the distance at which half of the population has died or gone to earth and they are not the same as the average distance flown which will be dealt with in the next section.

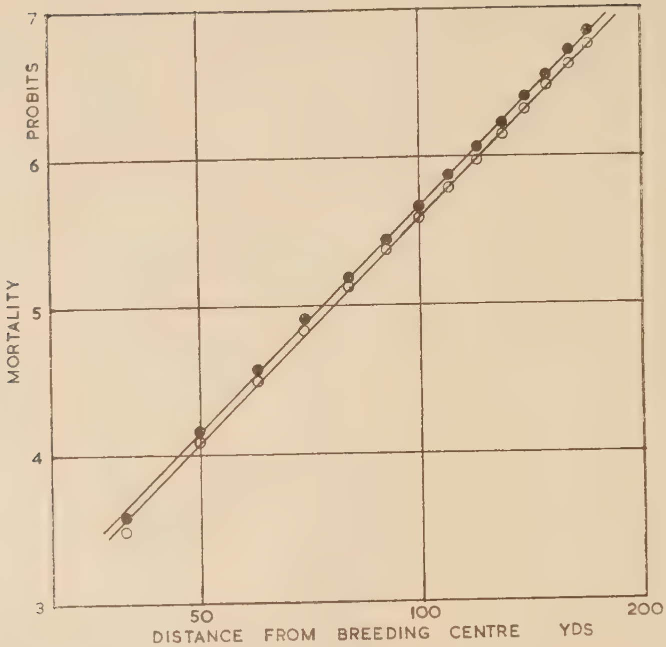

Fig. 13.—The relationship between the mortality of male (closed circles upper line) and female (open circles lower line) *C. impunctatus* and distance from the centre of breeding. Ordinate—percentage mortality in probits. Abscissa—distance from breeding centre in yards on a logarithmic scale.

(f) *Mean Flight Range of* C. impunctatus.

In Table XII it is shown that 632,561 $k\Delta x$ male *C. impunctatus* were found 25 yds. from the breeding place and 631,461 $k\Delta x$ were found 30 yds. away. Therefore (632,561 – 631,461) $k\Delta x$ males flew between 25 and 30 yds., that is 1,100 $k\Delta x$ males flew an average of 27·5 yds. This process can be repeated for different values of x. The sum of the products of the decrease in population size between x and $x+5$ (*i.e.*, $Nx - Nx+5$) and the mean distance flown by these individuals $(x+2\frac{1}{2})$ is the total distance flown by the entire population. The average distance flown can be obtained by dividing this sum by the number of individuals in the population (=632,561 $k\Delta x$ males). This calculation has been carried out for male and female *C. impunctatus* and the average distance flown by males is 79·0 yds. and by females 81·4 yds. (Tables XII and XIII).

The Vertical Distribution of *C. impunctatus.*

In the analysis of the vertical distribution of *C. impunctatus* the seasonal effect has been eliminated, as before, by expressing the weekly catch of each sex on each trap (2 ft., 6 ft. and 10 ft.) at each station as a percentage of the total weekly catch of male or female *C. impunctatus* at that station provided that the latter exceeded 10. As a result of this condition the number of observations varies from station to station and from week to week. In accordance with usual statistical practice the harmonic mean of the observations has been used in calculating the 95 per cent. fiducial intervals for stations and races. Where this interval indicates a significant difference between the races the exact error of the difference has been calculated in order to assess the correct probability of the occurrence of such a difference.

On account of the irregular shape of the woodland and the regular arrangement of the catching stations some woodland stations were actually sited on the moorland and *vice versa.* Thus BM4 was erected in the oak wood at Bannachra and will be considered along with the other oak wood stations, whilst stations WBW2 and WBW5 were placed in an open clearing in the woodland and WBW11 was sited in an exposed bracken covered situation.

(a) *In woodland.*

The most obvious difference between the locations of the woodland catching stations was that some were placed under tall oaks (Pl. X, fig. 3) and others amidst young birch saplings (Pl. X, fig. 4). The stations in the oak wood caught few *C. impunctatus* because they were relatively a long way from the breeding site. In order to make the best use of the data the stations have been amalgamated into two series and the results are presented in Table XIV. The differences between the two series are statistically insignificant and the vertical distribution of both sexes is approximately 10 per cent., 40 per cent. and 50 per cent. (In this section vertical distributions will always be given in the order 2 ft., 6 ft. and 10 ft.)

The birch woods at both Bannachra and Wester Bannachra were composed of either (1) dense thickets in which the herb layer was absent and the bare ground covered with decaying leaves or (2) a more open association of saplings with a herb layer of bracken or *Juncus articulatus.* There were eight stations located in the dense birch thickets. An analysis of variance was carried out for both sexes at each height and it was found that there were highly significant differences between the various stations (Tables XV and XVI). In every analysis except one (male *C. impunctatus* at 2 ft. when $P = 0 \cdot 04$) the probability that the observed differences were the result of experimental error was less than one chance in a thousand ($P < 0 \cdot 001$). The vertical distribution of the males varied from 13 per cent., 27 per cent., 60 per cent. at WBW7 to 18 per cent., 60 per cent., 22 per cent. at WBW6 and the female distribution from 15 per cent., 28 per cent., 57 per cent. at WBW7 to 17 per cent., 53 per cent., 30 per cent. at WBW6. These differences are all the more puzzling because of the apparently very similar nature of the environs of these stations.

Similarly, the analysis of variance of the vertical distribution of the six stations in the more open birch wood revealed highly significant differences between them with values of P for the males of insignificant, $<0 \cdot 001$, $<0 \cdot 001$, and for the females of $<0 \cdot 05$, $<0 \cdot 001$, $<0 \cdot 01$ (Tables XVII and XVIII). The degree of variation in the vertical distribution is also very similar ranging from 14 per cent., 26 per cent., 60 per cent. (BW1) to 15 per cent., 57 per cent., 28 per cent. (WBW10) in the males and 14 per cent., 36 per cent., 50 per cent. (WBW8) to 15 per cent., 55 per cent., 30 per cent. (WBW10) in the females. It appears therefore that the presence or absence of a herb layer under the birch saplings has little effect, if any, upon the vertical distribution of the adult *C. impunctatus,* and yet there must be some cause for the great differences between the stations.

The 14 birch-wood stations can be arranged in a series of decreasing catches on the 10-ft. and increasing catches on the 6-ft. trap. The vertical distributions of the first members of this series are statistically inseparable from those of the oak wood stations but the final members are statistically different. The 10-ft. traps at these birch-wood stations are among or near the lower branches of the saplings. Therefore if the leaf canopy acted as a barrier to the vertical migration of *C. impunctatus* the vertical distribution at a station would be profoundly modified by the exact spatial relationship between the leaf canopy and the 10-ft. trap. In the absence of any simpler alternative it seems likely that this is the explanation of the great variation in vertical distribution at the birch-wood stations but it is impossible to verify because of the difficulty inherent in obtaining an exact measurement of the height of the leaf canopy.

TABLE XIV.

Vertical distribution of C. *impunctatus* adults in the oak wood at Bannachra. Series A consists of BW4 and BM4 ; Series B of BW8, 9, 10 and 11. The results above the upper line refer to the spring race and those below the lower line to the summer race. The 95 per cent. fiducial limits of the mean are given for each group mean and the 95 per cent. fiducial interval for the difference between the spring and summer race means.

Week No.	Males						Females					
	2 ft.		6 ft.		10 ft.		2 ft.		6 ft.		10 ft.	
	A	B	A	B	A	B	A	B	A	B	A	B
3	6	0	32	17	62	83	13	—	45	—	42	—
4	16	19	40	44	45	37	33	11	36	47	31	42
5	15	21	17	32	69	47	12	15	16	35	72	50
6	8	18	46	35	46	45	3	17	37	24	60	59
7	9	0	42	53	48	47	9	0	50	27	41	73
8	4	5	38	37	58	58	14	16	36	47	50	37
9	7	7	31	40	62	53	7	6	28	40	65	54
10	11	11	43	39	46	50	11	15	43	39	46	46
11	9	11	43	62	49	26	13	8	35	51	52	41
12	25	15	29	35	46	50	28	21	36	29	36	50
Series mean ...	11·0	10·7	36·1	39·4	53·1	49·6	14·3	12·1	36·2	37·7	49·5	50·2
Group mean ...	10·9 ±2·3		37·8 ±3·9		51·4 ±4·6		13·3 ±3·5		36·9 ±5·1		49·8 ±5·7	
Spring race ...	12·9	4·8	32·9	8·4	54·3	9·8	14·9	7·7	34·3	11·2	50·9	13·4
Summer race ...	10·5		39·7		49·8		13·9		38·4		47·7	

In the oak wood the 10-ft. trap was far beneath the leaf canopy, which was at least 20 ft. above the ground (Pl. X., fig. 3). In this situation relatively few midges were caught on the 2-ft. trap, many more on the 6-ft. amd a few more on the 10-ft. trap (Table XIV). Since the environmental conditions are likely to be fairly uniform between the ground and the leaf canopy it was wondered if the midge density would go on increasing up to the leaf canopy or whether it would decrease after passing some critical height. Whilst there are no exact data available on this point there is some relevant information. Reasons have already been advanced for believing that the leaf canopy acts as a barrier to vertical migration. If this be true and if the *C. impunctatus* density increased or remained constant between 10 ft. above the ground and the leaf canopy then it would be anticipated that lowering the canopy would increase the midge density under it. Moreover, it would be expected that this would bring about not only an increase in the actual midge density but also an increase in the proportion of the population found immediately under the canopy. That is, lowering the canopy to 10 ft. would produce an increased percentage catch on the 10-ft. trap. But it has already been demonstrated that the percentage catch on the 10-ft. trap at the birch-wood stations, where the leaf canopy is about 10 ft. above the ground, is not significantly different from that on the 10-ft. trap at the oak-wood stations. Therefore lowering the leaf canopy from 20 ft. (oak wood) to 10 ft. (birch wood) has not increased the proportion of adults trapped at 10 ft. Has it increased the actual midge density ?

In fig. 11 the actual catch of *C. impunctatus* is plotted against the distance from the breeding centre. If the leaf canopy acted as a barrier to vertical migration and if an appreciable proportion of the adults was found above 10 ft. from the ground then the catch at the oak-wood stations would be determined by two factors (1) the distance from the breeding site and (2) the proportion of its population which occurred above 10 ft. from the ground. If this second factor were of any magnitude the oak-wood stations would catch less adults than expected. However, in fig. 11 the oak-wood stations are the five furthest away from the breeding site and of those one catches the expected number, two catch less and two catch more. So that there is no evidence from the actual total catches that any appreciable proportion of the population occurs above 10 ft.

In the absence of a shrub and herb layer under the oak trees, the environmental conditions between the ground and the leaf canopy are probably fairly uniform and are unlikely to be the reason for the scarcity of adult *C. impunctatus* in the 10–20 ft. zone. This implies that *C. impunctatus* has a self imposed " upper limit " to its vertical migration, which is independent of the environmental conditions.

(b) *On moorland.*

The vegetation on the moorland at Bannachra and Wester Bannachra is quite different. Bannachra moorland is covered with a luxuriant growth of bracken and contains a number of scattered trees (Pl. X, fig. 1 and Pl. XI, fig. 1) whereas Wester Bannachra moorland is treeless and has a variable herb layer of *Juncus* spp., coarse grasses and a small amount of bracken (Pl. X, fig. 2 and Pl. XI, fig. 2).

The analysis of the results obtained on Bannachra moorland revealed highly significant differences between the stations ; P having the following values—$<0{\cdot}001$; $<0{\cdot}01$; $<0{\cdot}001$ for males and $<0{\cdot}001$; insignificant ; $<0{\cdot}001$ for females (Tables XIX–XXII). This great difference was almost entirely due to one station BM6 which had a male vertical distribution of 4 per cent., 28 per cent., 68 per cent. compared with an average of 22 per cent., 39 per cent., 39 per cent. for the other stations and a female vertical distribution of 8 per cent., 32 per cent., 60 per cent., compared with 21 per cent., 38 per cent., 41 per cent. (*cf.* Tables XIX and XX with XXI and XXII). Moreover, the percentage catch of males on the 10-ft. trap at BM6 is significantly different from all the other stations and the catch on its 2-ft. trap is significantly different

TABLE XV.

Weekly percentage catch of male *C. impunctatus* on the 2-ft. trap and mean percentage catch on the 6-ft. and 10-ft. traps of the stations sited in the birch wood without a herb layer. The catch is expressed as a percentage of the total catch of male *C. impunctatus* at the station, providing that the latter exceeded 10. The observations above the upper line refer to the spring race and those below the lower line to the summer race. P_s and P_w are the probabilities that the variation between stations and between weeks might be due to experimental error. $t_{0\cdot05}s_{\bar{x}}$ is the 5 per cent. fiducial interval for the difference between the means. x, xx, xxx and xxxx difference between the races exceeds 5 per cent., 2 per cent., 1 per cent. and 0·1 per cent. level of significance.

Week No.	W.B.W.1	W.B.W.3	W.B.W.6	W.B.W.7	B.W.2	B.W.5	B.W.6	B.W.7	Weekly Mean	
2	0	0	33	—	—	—	—	—	11·0	
3	10	14	39	10	7	12	—	—	15·3	
4	—	—	—	—	17	21	16	13	16·7	
5	10	20	22	15	—	12	—	15	15·6	
6	14	15	27	22	8	15	—	11	16·0	
7	10	13	16	15	4	11	4	—	10·4	
8	8	16	16	23	14	33	0	22	16·5	
9	8	13	15	15	5	8	—	10	10·5	
10	14	9	15	14	8	19	21	17	14·6	
11	9	5	12	7	10	9	5	15	9·0	
12	13	15	12	14	12	21	5	10	12·7	
13	12	7	5	4	—	—	—	—	7·0	
14	20	11	4	2	—	—	—	—	9·2	
Station mean	10·7	11·5	18·0	12·8	9·4	16·1	8·5	14·1	$t_{0\cdot05}s_{\bar{x}}$ 5·9	
Spring race mean	6·7	11·3	xxxx 31·3	12·5	10·6	15·0	16·0	13·0	9·6	
Summer race mean	11·8	11·1	xxxx 11·9	11·8	9·8	18·0	7·8	14·8		P_s 0·04 P_w insignificant
6 ft. Station mean	41·6	32·8	59·8	27·0	47·1	53·1	40·2	41·6	10·3	P <0·001
6 ft. Spring race mean ...	37·0	34·6	53·0	31·0	46·3	61·0	33·0	46·0	14·5	P_w insignificant
6 ft. Summer race mean ...	41·5	33·7	64·2	24·6	45·6	47·4	44·5	38·8		
ft. Station mean	47·8	55·7	22·2	60·2	43·6	30·7	51·3	44·4	8·8	P_s <0·001
ft. Spring race mean ...	56·3	54·0	15·6	56·5	43·3	23·7	51·0	41·0	14·2	P_w insignificant
ft. Summer race mean ...	46·7	55·1	23·8	63·6	44·6	34·6	48·0	46·4		

TABLE XVI.

Weekly percentage catch of female *C. impunctatus* on the 2-ft. trap and mean percentage catch on the 6-ft., and 10-ft. traps of the stations sited in the birch wood without a herb layer. For other details see Table XV.

	Week No.	W.B.W.1	W.B.W.3	W.B.W.6	W.B.W.7	B.W.2	B.W.5	B.W.6	B.W.7	Weekly Mean	
	3	4	22	35	21	12	36	—	27	22·4	
	4	25	—	—	—	26	28	13	12	20·8	
	5	20	23	27	6	—	38	—	18	22·1	
	6	19	26	22	26	12	36	15	21	22·1	
	7	20	20	15	19	19	25	17	—	19·2	
	8	14	16	19	21	5	25	—	22	15·2	
	9	7	9	15	21	7	22	32	28	17·6	
	10	15	11	18	13	4	17	15	—	11·6	
	11	5	5	10	12	10	20	11	23	12·0	
	12	22	22	11	15	6	25	10	19	16·2	
	13	22	16	11	9	—	—	—	—	14·5	
	14	18	14	6	9	—		—		11·7	
	Station mean	15.9	16·7	17·2	15·6	11·2	27·3	14·1	18·9	$t_{0{\cdot}05}s_{\bar{d}}$ 5·5	P_s <0·001
	Spring race mean...	16·3	22·5	31·0^{xxxx}	13·5	16·7^{x}	34·8^{xxx}	14·0	19·5	8·9	P_w <0·01
	Summer race mean	15·4	14·1	13·1^{xxxx}	14·9	6·4^{x}	21·8^{xxx}	13·6	18·4		
6 ft.	Station mean	37·8	37·2	52·5	27·7	46·0	42·0	46·8	35·7	8·2	P_s =0·001
	Spring race mean	30·3	42·0	53·5	31·0	45·3	43·0	31·0^{xxx}	44·7^{xx}	13·4	P_w insignificant
	Summer race mean	39·3	36·2	56·0	25·8	48·4	46·2	55·8^{xxx}	28·4^{xx}		
10 ft.	Station mean	46·3	46·1	30·3	56·6	42·7	30·5	39·2	45·4	11·5	P_s <0·001
	Spring race mean	53·3	35·5	25·5	55·5	38·0	21·7	55·0^{x}	35·7	18·7	P_w insignificant
	Summer race mean	45·2	49·6	30·8	59·2	45·2	32·0	30·8^{x}	53·2		

TABLE XVII.

Weekly percentage catch of male *C. impunctatus* on the 2-ft. trap and the mean percentage catch on the 6-ft. and 10-ft. traps of the stations sited in the birch wood with a herb layer. For other details see Table XV.

	Week No.	W.B.W.4	W.B.W.8	W.B.W.9	W.B.W.10	B.W.1	B.W.3	Weekly Mean	
	2	0	—	—	17	—	—	8·5	
	3	31	12	24	16	14	17	19·0	
	4	—	10	—	20	26	0	14·0	
	5	23	15	—	—	9	12	14·7	
	6	11	11	22	19	0	2	10·8	
	7	12	20	15	20	29	8	17·3	
	8	7	14	14	16	5	—	11·2	
	9	3	20	18	9	22	11	13·8	
	10	17	13	11	12	15	22	15·0	
	11	5	11	0	6	12	12	7·6	
	12	26	18	13	12	9	24	17·0	
	13	13	21	14	14	—	—	16·5	
	14	—	14	0	14	—	—	9·0	
	Station mean	13·5	14·9	13·1	14·5	14·1	12·0	$t_{0\cdot05}s_d$ 6·1	P_s insignificant
	Spring race mean	13·8						4·0	P_w insignificant
	Summer race mean	13·3							
6 ft.	Station mean	34·8	38·9	34·7	57·3	25·8	38·9	8·6	$P_s < 0\cdot001$
	Spring race mean	33·3	41·6	29·0	54·3	27·0	41·7	13·7	P_w insignificant
	Summer race mean	33·8	37·8	35·8	57·1	23·0	47·5		
10 ft.	Station mean	51·6	46·2	52·2	28·2	60·0	49·0	10·6	$P_s < 0\cdot001$
	Spring race mean	48·7	46·0	47·0	24·7	61·0	50·7	17·0	P_w insignificant
	Summer race mean	54·1	45·7	53·5	30·1	64·2	45·0		

TABLE XVIII.

Weekly percentage catch of female *C. impunctatus* on the 2-ft. trap and the mean percentage catch on the 6-ft. and 10-ft. traps of the stations sited in the birch wood with a herb layer. For other details see Table XV.

Week No.		W.B.W.4	W.B.W.8	W.B.W.9	W.B.W.10	B.W.1	B.W.3	Weekly Mean	
3		26	16	24	23	33	17	23·1	
4		—	11	—	21	26	0	14·5	
5		31	21	—	—	16	3	17·7	
6		6	7	30	19	19	7	14·6	
7		12	19	37	13	30	18	22·5	
8		17	22	35	31	33	—	27·6	
9		10	16	19	10	16	18	14·8	
10		22	11	10	8	22	24	16·1	
11		9	8	26	7	18	15	13·8	
12		35	21	7	10	15	33	20·1	
13		18	10	18	7	—	—	13·2	
14		—	10	—	14	—	—	12·0	
Station mean		18·6	14·3	22·9	14·8	22·8	15·0	$t_{0\cdot05}s_{\bar{d}}$ 7·1	P_s =0·05
Spring race mean		28·5	16·0	24·0	22·0	23·5	xxx 6·8	11·9	P_w insignificant
Summer race mean		17·6	14·3	21·7	12·5	20·8	xxx 22·5		
6 ft.	Station mean	36·3	35·8	36·0	54·6	34·0	43·4	8·9	P_s <0·001
	Spring race mean	28·5	35·0	29·0	47·0	28·0	44·3	14·8	P_w =0·03
	Summer race mean	35·2	26·5	38·6	57·8	37·6	37·5		
10 ft.	Station mean	45·1	50·0	41·1	30·5	43·6	41·4	8·8	P_s <0·01
	Spring race mean	43·0	49·0	47·0	31·0	49·0	48·8	14·6	P_w =0·03
	Summer race mean	47·1	48·9	39·7	26·4	42·0	40·0		

from all the others except BM9. Similarly the percentage catch of females on the 10-ft. trap at BM6 is significantly different from all others except BM3. Station BM6 shows a number of other peculiar features. It has the highest catch of *C. impunctatus* on Bannachra moorland and therefore is presumably located near the breeding place. By analogy with BW1 and BW2 it would be expected that the breeding site would be S. or W. of BM6 since the latter catches many adults and the prevailing wind is SW., but the area to the S. and W. of BM6 is a dry bracken covered slope, which appears to be most unsuitable for midge breeding. Judging from the flora, the probable breeding place is NE. of the station and in support of this it was observed that there were noticeably more midges trapped on the sections of the traps facing the N. and E. than on the S. and W. sectors, although no quantitative data were collected. It would appear, therefore, that the midges were moving outwards against the prevailing wind. A more likely explanation is that as a result of the SW. wind coming over the pine wood there is created a NE. current of air at ground level which brings the midges to BM6 from the breeding place. At BM6 there is a group of trees which acts as an obstacle to the ground current causing it to rise and in so doing it carries the midges upwards thus producing the unusually high vertical distribution which was found in both sexes of *C. impunctatus* at station BM6.

The remaining stations on Bannachra moorland form a more or less homogeneous group (Tables XXI and XXII). The probabilities that the observed differences between the stations are significant are 0·05, 0·04, 0·04 for the males and $<0{\cdot}01$, insignificant, 0·04 for the females. The vertical distribution of the males is 22 per cent., 39 per cent., 39 per cent., and of the females 21 per cent., 38 per cent., 41 per cent., although the variation among the females on the 2-ft. traps is greater than that due to experimental error.

On Wester Bannachra moorland the analysis of the vertical distribution of *C. impunctatus* produced the following probabilities that the observed differences were due to experimental error $<0{\cdot}001$, insignificant, 0·04 for males and $<0{\cdot}001$, $<0{\cdot}001$, $<0{\cdot}01$ for females (Tables XXIII and XXIV). Clearly there are very real differences between the stations. The most obvious topographical differences between the stations concern the nature of the surrounding vegetation. The stations could be divided into four groups: (1) stations fully exposed to the prevailing wind and surrounded by shallow vegetation not more than 6 ins. in height, (2) stations in small open clearings about 6 ft. diameter surrounded by vegetation about 2 ft. in height or more, (3) stations surrounded by *Juncus* sp., and (4) stations surrounded by bracken. When the analysis is repeated with the male catches at 2 ft., it is found that the within group variance is insignificant but the between group variance is highly significant, Table XXIII. Therefore, the observed differences between the percentage catches of males on the 2-ft. traps are solely related to the vegetative cover at the station, there being less males amongst the bracken and more among the *Juncus* sp. and in sheltered clearings. It is of interest that the vertical distribution of *C. impunctatus* at the stations among the bracken (Group 4) agrees very closely with that obtained on Bannachra moorland, 23 per cent., 37 per cent., 40 per cent. compared with 22 per cent., 39 per cent., 39 per cent.

When the same group analysis is carried out for the females on the 2-ft. traps, the variation within the groups is still significant ($P=0{\cdot}02$) but most of the variation is attributable to differences between the groups ($P<0{\cdot}001$) (Table XXIV). However, when the group analysis is applied to the female catch at 6 ft., the within group variance is very highly significant ($P<0{\cdot}001$) and the between group variance of little significance ($P=0{\cdot}05$) (Table XXIV). Clearly some other factor is operative here, but its nature is not known. It will be recalled that in the analysis of the vertical distribution of *C. impunctatus* on Bannachra moorland the females showed greater variation than the males.

It appears therefore that the vertical distribution of *C. impunctatus*, when it is fully exposed to the prevailing wind, is 32 per cent., 31 per cent., 37 per cent. (male) and 28 per cent., 33 per cent., 39 per cent. (female) (Group 1). This can be compared with the vertical distribution of *C. impunctatus* under the sheltered conditions of the oak wood when its distribution was 11 per cent., 38 per cent., 51 per cent. (male) and 13 per cent., 37 per cent., 50 per cent. (female) (Table XIV). The effect of exposure to wind is to reduce the average height of flying from 7·6 ft. to 6·2 ft. for males and from 7·5 ft. to 6·4 ft. for females. In this connection it is interesting to note that station WBW5 (Tables XIX and XX) is situated under intermediate topographical conditions (Pl. XI, fig. 3). Its ground flora is similar to Group 1 but it is placed in a sheltered clearing in the woods, and in accordance with its intermediate environment it has an intermediate vertical distribution and average flight height—22 per cent., 44 per cent., 34 per cent. (6·5 ft., males) and 18 per cent., 43 per cent., 39 per cent. (6·8 ft., females).

When shelter is either provided by clearings among the herb layer or by *Juncus* sp. (Groups 2 and 3) there is a tendency for the adults to congregate among it so that they record vertical distributions of 42 per cent., 28 per cent., 30 per cent. (male) and 35 per cent., 33 per cent., 32 per cent.(female) compared with distributions of 32 per cent., 31 per cent., 37 per cent. (male) and 28 per cent., 33 per cent., 39 per cent. (female) at fully exposed stations. Station WBW2 is sited in a *Juncus* swamp in a woodland clearing (Pl. XI, fig. 3) and it would be expected that its vertical distributions would be intermediate between Groups 2 and 3 and that of the true woodland stations. Instead, however, it records distributions of 46 per cent., 35 per cent., 19 per cent. (male) and 40 per cent., 35 per cent., 25 per cent. (female) (Tables XIX and XX), which are lower than the average for Groups 2 and 3 but very similar to those of station WBM10 in Group 2 (Tables XXIII and XXIV).

The shelter provided by bracken appears to be less attractive to *C. impunctatus* because there is a decrease in the catch on the 2 ft. trap. The bracken stations record vertical distributions of 23 per cent., 37 per cent., 40 per cent. (male) and 20 per cent., 40 per cent., 40 per cent. (female) compared with those of 39 per cent., 30 per cent., 31 per cent. (male) and 37 per cent., 35 per cent., 28 per cent. (female) among *Juncus* sp. From this it would appear that most of the *C. impunctatus* activity takes place above the bracken and not amidst the shelter of its fronds. This may be related to the structure of the bracken, the horizontal pinnae of which would interfere with vertical migration whereas the upright *Juncus* stems would provide no such obstacle. There is very close agreement between the vertical distributions of Group 4 and those at Bannachra moorland (22 per cent., 39 per cent., 39 per cent., male, and 21 per cent., 38 per cent., 41 per cent. female) which is also bracken covered. However, station WBW11 is sited amongst bracken at the exposed boundary of the woodland and has vertical distributions of 35 per cent., 40 per cent., 25 per cent. (male) and 31 per cent., 42 per cent., 27 per cent. (female) (Tables XIX and XX). Although these differ considerably from the means of the bracken stations, they are fairly similar to those recorded at station BM5 (31 per cent., 34 per cent., 34 per cent. (male) and 31 per cent., 40 per cent., 29 per cent. (female), (Tables XXI and XXII).

(c) *Vertical distribution of sexes.*

The mean percentage catches of male and female *C. impunctatus* under different conditions are given in Table XXV. There is only a very small difference in the vertical distribution of the sexes. In the woodland the males are slightly more numerous on the 10-ft. trap and the females correspondingly more abundant on the 2-ft. trap, but these differences are only detectable in very large samples. On Bannachra moorland there is no difference between the sexes whilst on Wester Bannachra moorland although the males are more abundant on the 2-ft. trap and the females on the 6-ft. trap there is no difference between the sexes on the 10-ft. trap.

TABLE XIX.

Vertical distribution of male C. *impunctatus* at station W.B.W.2, 5 and 11, and B.M.6. For other details see Table XV.

Week No.	W.B.W.2			W.B.W.5			W.B.W.11			B.M.6		
	2 ft.	6. ft.	10 ft.	2 ft.	6 ft.	10 ft.	2 ft.	6 ft.	10 ft.	2 ft.	6 ft.	10 ft.
2...	46	27	27	—	—	—	41	47	12	—	—	—
3...	50	34	16	28	48	24	38	44	18	5	43	52
4...	35	35	30	26	16	58	—	—	—	—	—	—
5...	—	—	—	—	—	—	34	44	22	11	28	61
6...	44	37	19	12	49	39	37	41	22	3	32	65
7...	61	21	18	24	49	27	29	37	34	8	26	66
8...	60	27	13	17	46	37	54	34	12	2	26	73
9...	37	46	17	27	41	32	33	41	26	3	23	74
10...	39	36	25	27	38	35	31	43	26	6	26	68
11...	43	40	17	21	56	23	34	32	34	2	28	70
12...	35	47	18	28	41	31	20	43	37	2	18	80
13...	49	37	14	17	55	28	31	36	33	—	—	—
14...	50	38	12	16	42	42	—	—	—	—	—	—
Mean	45·8	35·4	18·8	22·1	43·7	34·2	34·7	40·2	25·1	4·4	27·7	67·7
Spring race	43·7	32·0	24·3	27·0	32·0	41·0	37·7	45·0	17·3	6·8	32·2	61·0
Summer race	46·8	36·5	16·7	22·1	46·0	31·9	33·1	38·0	28·9	3·3	23·7	73·0

TABLE XX.

Vertical distribution of female *C. impunctatus* at stations W.B.W.2, 5 and 11, and B.M.6. For other details see Table XV.

Week No.	W.B.W.2			W.B.W.5			W.B.W.11			B.M.6		
	2 ft.	6 ft.	10 ft.	2 ft.	6 ft.	10 ft.	2 ft.	6 ft.	10 ft.	2 ft.	6 ft.	10 ft.
2	—	—	—	—	—	—	—	—	—	—	—	—
3	30	50	20	17	44	39	37	45	18	9	27	64
4	39	34	27	33	27	40	—	—	—	—	—	—
5	—	—	—	—	—	—	35	43	22	17	67	17
6	35	17	48	11	50	39	29	48	23	13	30	58
7	46	33	21	22	37	41	24	42	34	12	25	63
8	67	20	13	19	33	48	48	32	20	2	25	73
9	42	28	30	18	48	34	25	49	26	6	25	69
10	30	46	24	13	39	48	38	38	24	6	36	58
11	41	35	24	24	30	46	27	39	34	3	25	72
12	32	29	39	15	41	44	19	41	40	4	32	64
13	41	38	21	13	52	35	28	39	33	—	—	—
14	39	57	4	16	68	16	—	—	—	—	—	—
Mean	40·2	35·2	24·6	18·3	42·6	39·1	31·0	41·6	27·4	8·0	32·4	59·8
Spring race	34·5	42·0	23·5	25·0	35·5	39·5	36·0	44·0	20·0	13·0	41·3	46·3
Summer race	42·3	35·8	22·0	17·5	43·5	39·0	29·9	40·0	30·1	4·2	28·6	67·2

TABLE XXI.

Weekly percentage catch of male *C. impunctatus* on the 2-ft. trap and the mean percentage on the 6-ft. and 10-ft. traps of the stations on Bannachra moorland. For other details see Table XV.

Week No.	B.M.1	B.M.2	B.M.3	B.M.5	B.M.7	B.M.8	B.M.9	B.M.10	B.M.11	Weekly Mean	
3	—	—	—	38	38	28	14	—	27	29·0	
4	43	—	20	34	24	—	25	13	—	26·5	
5	—	—	—	—	8	33	—	18	13	18·0	
6	33	0	—	18	7	30	12	36	0	19·4	
7	0	—	0	41	9	48	0	27	0	15·6	
8	—	—	—	25	12	13	18	—	21	17·8	
9	24	—	20	29	7	11	20	23	21	19·4	
10	23	42	24	29	25	37	16	37	22	28·4	
11	20	12	14	23	14	13	22	24	21	18·1	
12	25	—	23	45	39	23	9	46	18	28·5	
Station mean	24·0	18·0	16·8	31·3	18·3	26·2	15·1	28·0	15·9	$t_{0\cdot05}s_{\bar{d}}$ 11·3	
Group mean					21·8 ±2·7						P = 0·05
Spring race mean					20·5					5·5	P_w insignificant
Summer race mean					23·5						
6 ft. Station mean	42·3	41·3	42·2	34·0	29·5	40·6	50·6	33·0	44·3	12·7	
6 ft. Group mean					39·4 ±3·0						P = 0·04
6 ft. Spring race mean					36·6					6·2	P_w insignificant
6 ft. Summer race mean					42·4						
10 ft. Station mean	33·6	40·7	41·0	34·3	51·8	32·9	34·2	39·1	39·4	12·3	
10 ft. Group mean					38·6 ±2·9						P_s = 0·04
10 ft. Spring race mean					xxx 42·6					6·0	P_w = 0·03
10 ft. Summer race mean					xxx 34·3						

TABLE XXII.

Weekly percentage catch of female *C. impunctatus* on the 2-ft. trap and the mean percentage catch on the 6-ft. and 10-ft. traps of the stations on Bannachra moorland. For other details see Table XV.

Week No.	B.M.1	B.M.2	B.M.3	B.M.5	B.M.7	B.M.8	B.M.9	B.M.10	B.M.11	Weekly Mean	
3	—	—	—	27	58	—	9	—	—	31·3	
4	33	—	17	28	34	—	17	21	—	25·0	
5	—	0	—	—	21	46	—	17	—	21·0	
6	6	—	—	38	17	23	8	29	—	20·2	
7	—	—	—	29	19	32	0	40	0	20·0	
8	—	—	—	25	12	—	—	—	6	14·3	
9	29	20	10	41	4	10	7	8	24	19·1	
10	24	24	28	29	22	24	10	42	11	26·8	
11	13	17	17	22	16	15	10	37	23	21·3	
12	5	23	14	36	30	33	8	30	17	24·5	
Station mean	18·3	16·8	17·2	30·6	23·3	26·1	8·6	28·0	13·5	$t_{0\cdot05}s_{\bar{d}}$ 8·0	$P_s < 0\cdot01$
Spring race mean	19·5	0·0	17·0	31·0	32·5	34·5	11·3	22·3	—	16·9	P_w insignificant
Summer race mean	17·8	21·0	17·3	30·6	16·8	20·5	8·8	29·3	13·5		
6 ft. Station mean	45·8	39·0	27·4	40·2	31·0	41·6	47·0	32·8	41·2	13·9	
6 ft. Group mean	38·5 ±3·3										P_s insignificant
6 ft. Spring race mean ...	37·5									7·3	P_s insignificant
6 ft. Summer race mean ...	38·6										
10 ft. Station mean	36·0	44·0	55·8	29·0	45·4	32·1	44·6	39·4	45·2	14·6	
10 ft. Group mean	40·6 ±3·5										$P_s = 0\cdot04$
10 ft. Spring race mean ...	39·3									7·7	P_w insignificant
10 ft. Summer race mean ...	41·4										

TABLE XXIII.

Weekly percentage catch of male *C. impunctatus* on the 2-ft. trap and the mean percentage catch on the 6-ft. and 10-ft. traps of the stations on Wester Bannachra moorland. For definitions of groups see text. For other details see Table XV.

	Week No.	GROUP 1				GROUP 2			GROUP 3		GROUP 4		Weekly Mean
		W.B.M.2	W.B.M.3	W.B.M.5	W.B.M.7	W.B.M.8	W.B.M.10	W.B.M.11	W.B.M.1	W.B.M.9	W.B.M.4	W.B.M.6	
	3	30	29	50	24	31	59	15	46	31	34	11	32·7
	4	32	20	16	55	—	42	43	25	31	17	33	31·4
	5	13	—	12	—	21	—	6	—	—	11	29	16·2
	6	36	35	16	36	13	52	54	21	17	17	14	28·3
	7	10	43	0	—	—	30	79	65	21	31	20	33·2
	8	18	36	26	8	23	67	40	54	32	36	47	35·2
	9	30	36	18	17	11	62	40	26	61	28	40	33·5
	10	35	37	20	32	38	50	58	31	62	13	17	35·7
	11	50	20	41	30	55	71	76	25	59	22	25	43·1
	12	55	48	27	0	64	24	31	38	49	20	16	32·9
	13	48	31	64	27	68	52	55	31	51	28	17	42·9
	14	55	53	55	47	22	42	44	41	32	14	16	38·3
	Station mean	34·8	35·3	28·8	27·6	34·6	50·1	45·1	36·6	40·5	22·6	22·9	$t_{0\cdot05}\,S_{\bar{d}}$ 13·4
	Group mean	31·7 ±4·6				43·6 ±5·3			38·6 ±6·5		22·8 ±6·2		P (between groups) <0·001.
	Spring race mean ...	29·2	10·0			x 33·6	11·7		28·5	14·9	20·7	13·5	P (within groups) insignificant.
	Summer race mean ...	34·4				x 47·2			42·2		24·2		P_w insignificant
6 ft.	Station mean ...	28·5	32·4	31·4	30·2	27·2	28·1	24·4	33·2	27·5	37·8	35·6	9·0
	Group mean ...	30·6 ±3·1				26·5 ±3·6			30·4 ±4·4		36·7 ±4·2		P_s insignificant
	Spring race mean	29·0	6·8			31·9	7·9		30·1	10·1	41·5	9·2	P_w insignificant
	Summer race mean	30·5				24·1			30·1		34·0		
10 ft.	Station mean ...	36·6	31·3	40·3	42·1	38·1	21·9	30·5	30·0	31·9	39·8	41·3	12·9
	Group mean ...	37·5 ±4·2				29·9 ±4·9			31·0 ±6·0		40·5 ±5·6		P_s=0·04
	Spring race mean	41·7	9·3			34·2	10·9		x 41·1	13·8	37·6	12·5	P_w=0·02
	Summer race mean	34·5				28·7			x 26·7		42·4		

Weekly percentage catch of female *C. impunctatus* on the 2-ft. trap and the mean percentage
stations on Wester Bannachra moorland. For definitions of groups see text. For other details see Table XV.

	Week No.	GROUP 1				GROUP 2			GROUP 3		GROUP 4		Weekly Mean
		W.B.M.2	W.B.M.3	W.B.M.5	W.B.M.7	W.B.M.8	W.B.M.10	W.B.M.11	W.B.M.1	W.B.M.9	W.B.M.4	W.B.M.6	
	3	30	27	32	26	20	19	—	53	—	15	23	27·2
	4	31	8	32	53	22	42	36	47	33	20	31	32·3
	5	13	—	3	—	20	—	11	—	—	11	24	13·7
	6	25	9	38	50	0	41	36	12	30	37	23	27·4
	7	—	20	—	—	—	33	25	40	15	14	—	24·5
	8	7	25	6	—	50	54	22	—	38	17	44	29·2
	9	22	24	16	27	12	44	23	17	47	15	17	24·0
	10	45	22	28	31	32	43	33	29	59	20	24	33·3
	11	54	27	31	32	34	45	54	25	57	15	14	35·3
	12	22	28	31	10	46	33	32	16	72	21	13	29·5
	13	45	28	51	27	58	25	50	22	55	20	14	35·9
	14	46	21	41	37	44	33	35	18	49	16	20	32·7
	Station mean	30·9	21·7	28·1	32·6	30·7	37·5	32·5	27·9	43·5	18·4	22·5	$t_{0\cdot05}s_{\bar{x}}$ 7·6
	Group mean	28·1 ±3·7				35·5 ±4·4			36·7 ±5·4		20·3 ±5·1		P (between groups) <0·001
	Spring race mean ...	26·9 } 8·0				xxx 24·7 } 9·3			35·0 } 12·7		23·0 } 10·7		P (within groups) =0·02
	Summer race mean ...	29·0				38·2			38·8		19·3		P_w =0·05
6 ft.	Station mean ...	26·8	42·8	30·7	30·0	27·5	34·8	32·3	43·3	27·5	38·9	41·2	8·8
	Group mean ...	32·7 ±3·1				31·6 ±3·6			35·4 ±4·6		40·0 ±4·3		P (between groups) <0·05
	Spring race mean	28·5	52·3	33·8	24·3 } 14·0	31·6 } 9·4			33·0	35·5 } 14·0	37·8 } 9·1		P (within groups) <0·001
	Summer race mean	25·9	41·0	29·0	32·8	30·0			45·7	25·6	43·1		P_w insignificant
10 ft.	Station mean ...	42·0	35·5	41·3	37·8	41·7	27·8	35·4	28·8	27·1	42·5	36·5	10·3
	Group mean ...	39·2 ±3·7				35·0 ±4·1			28·0 ±5·3		39·6 ±5·0		P (between groups) <0·01
	Spring race mean	38·9 } 7·8				xx 43·9 } 9·1			31·2 } 12·5		39·5 } 10·5		P (within groups) insignificant
	Summer race mean	38·9				xx 31·8			26·4		38·1		P_w <0·01

TABLE XXV.

Average percentage catch of male and female *C. impunctatus* under different conditions. xx, xxx and xxxx indicate, respectively, when the difference between the sexes exceeds the 2 per cent., 1 per cent. and 0·1 per cent. level of significance.

Height	Oak Wood		Birch Wood with Herb Layer		Birch Wood no Herb Layer		Wester Bannachra Moorland		Bannachra Moorland	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
10 ft.	51·4 ±4·6	49·8 ±5·7	47·2 ±3·0	xx 42·0 ±2·6	44·1 ±2·2	42·3 ±2·8	35·0 ±2·7	36·2 ±2·2	38·6 ±2·9	40·5 ±3·5
6 ft.	37·8 ±3·9	36·9 ±5·1	39·0 ±2·4	40·1 ±2·6	43·0 ±2·2	40·4 ±2·0	30·7 ±1·9	xx 34·2 ±1·9	39·4 ±3·0	38·5 ±3·3
2 ft.	10·9 ±2·3	13·3 ±3·5	13·8 ±1·8	xxx 17·9 ±2·0	12·9 ±1·4	xxxx 17·2 ±1·4	34·3 ±2·8	xx 29·6 ±2·2	21·8 ±2·7	21·0 ±2·7

(d) *Vertical distribution of races.*

In Tables XIV to XXIV the percentage catch of each race at each height is given together with the 95 per cent. fiducial interval. Where the differences between stations are insignificant the observations on each race have been pooled. In all, there are 126 comparisons between the two races and the expected and observed frequencies with which certain levels of significance are exceeded are—

No. of Observation ...	$P<0{\cdot}05$	$P<0{\cdot}02$	$P<0{\cdot}01$	$P<0{\cdot}001$
Expected	6·3	2·5	1·3	0·13
Observed	13	9	7	2

The observed frequencies are markedly larger than those expected and hence there is probably a real difference in the vertical distributions of the two races. However, this difference is not necessarily due to the two races but may be related to seasonal changes in the environment since the two races do not coexist. Differences due to experimental error are unlikely to occur in both sexes at the same station or group of stations but environmental differences are likely to be revealed in both sexes. Station WBW6 and Group 2 of the Wester Bannachra moorland stations show differences in both sexes. At WBW6 the summer race shows a lower catch (13 per cent.) on the 2-ft. trap than the spring race (31 per cent.). This station is a birch wood station with its highest catch on the 6-ft. trap. This would indicate that the leaf canopy is low and therefore it would be anticipated that the unfolding of the foliage would lead to a lower distribution of adults and not a higher one as observed.

The three stations in Group 2 on Wester Bannachra moorland show an increased catch of the summer race on the 2-ft. trap. These stations are situated in small clearings in the herb layer which have been shown to be attractive to adult *C. impunctatus*. Therefore it would be expected that as the herb layer became more dense and the clearing more sheltered the adults would be more attracted. This would explain the observed increased catch on the 2-ft. trap.

The observed differences between the races may in part be due to seasonal changes in the environment and partly to real differences in behaviour of the two races.

Summary.

Weekly observations on two populations of *Culicoides impunctatus* Goet. were made in 1948 at Bannachra and Wester Bannachra, near Loch Lomond, throughout the whole of the *C. impunctatus* season by means of " sticky " traps. Each site had an area of 5 acres of which half was composed of woodland and half of moorland. Twenty-two catching stations, each consisting of three traps placed 2 ft., 6 ft., and 10 ft. above the ground were erected on each site, equal numbers being placed in the woodland and moorland. There were therefore 132 traps to be changed weekly and a total of 67,033 *Culicoides* were trapped of which 65,576 (97·9 per cent.) were *C. impunctatus*. In 1949 observations were repeated only in Bannachra woodland.

The results have been analysed in the light of two alternatives (1) that *C. impunctatus* is a single biological entity and (2) that *C. impunctatus* is composed of two biological races.

When the seasonal effect was eliminated the stations showed very real differences between their weekly catches. Some stations recorded a large catch every week whilst others always recorded low catches. The total seasonal catch at each station was plotted on plans of the two sites and it then became apparent that there were two centres of high *C. impunctatus* density at Bannachra, one in the woodland and the other on the moorland, whilst at Wester Bannachra there were two foci in the woodland and a very diffuse zone of high midge density on the moorland. It has been shown that the centre of high midge density in Bannachra woodland was associated with a concentrated breeding place which supplied all the adults for the

woodland ($2\frac{1}{2}$ acres) and moreover this centre remained unaltered in 1949. The importance of these observations on the control of *C. impunctatus* is discussed.

After the breeding place in Bannachra woods was discovered it became clear that the majority of adult *C. impunctatus* moved in an eastward direction from the breeding place and very few went westwards. This was attributed to the influence of the prevailing SW. wind. Knowing the centre of breeding in Bannachra woods it was possible to calculate the regression of the midge density on the distance from the breeding site. It was found that there was a linear relationship between the logarithm of the catch and the distance from the breeding site. A useful statistic is the reciprocal of the regression which may be designated as the density coefficient. It is the distance at which the density has decreased to 1/10th of its initial value. The density coefficients for male and female *C. impunctatus* are 62·5 yards and 64·9 yards respectively. An attempt has been made to separate the effects of " dilution " and " mortality " on the density of *C. impunctatus*.

The mortality of male and female *C. impunctatus* is shown to be normally distributed with respect to the logarithm of the distance flown. These distributions are male 1·867±0·197 (=73·6 yds. ; 46·8–115·9 yds.) and female 1·885±0·197 (=76·7 yds. ; 48·8–120·8 yds.).

From the density/distance formula the average distances flown by male and female *C. impunctatus* have been calculated. They are 79·0 yards (males) and 81·4 yards (females).

The analysis of the vertical distribution of *C. impunctatus* revealed that each station had its own definite pattern. The stations sited amidst the birch saplings showed very real differences between themselves which were not related to the ground flora. It is suggested that these are related to the height of the leaf canopy. Evidence was also produced which pointed to the fact that in the oak wood where the base of the leaf canopy was about 20 ft. from the ground, *C. impunctatus* had a self-imposed " ceiling " of little more than 10 ft.

The ground flora was found to have an appreciable effect upon the vertical distribution of *C. impunctatus* on the moorland. Thus where the ground flora provided only very shallow shelter, *i.e.*, about 6 ins. in depth, equal numbers of adults were captured at each height, but when the stations were sited amongst *Juncus* sp. or in small sheltered clearings in the herb layer then more adults were caught on the 2-ft. trap. However, the shelter provided by bracken *Pteridium aquilinum* appeared to be less attractive and there were fewer adults caught on the 2-ft. traps at stations sited in dense bracken than at other moorland stations.

In the woodland the females were slightly more abundant on the 2-ft. trap and the males more numerous on the 10-ft. trap but the differences in distribution, although significant, were small. On the moorland the males were more abundant at 2 ft. and the females at 6 ft.

Although the general pattern was very similar there were highly significant differences between the horizontal and vertical distributions of the two races of *C. impunctatus*.

Acknowledgements.

I wish to record my thanks to Professor C. M. Yonge, F.R.S., for giving me the full use of his Department at the University of Glasgow and at the University Field Station at Rossdhu, Loch Lomond ; to Mr. J. R. Lumsden, of Arden, for allowing these experiments to be carried out on his estate at Bannachra ; to my assistant Mr. D. G. R. Sangster for being all that an assistant should be and to Mr. M. V. Brian for reading and criticising the manuscript. The work was done whilst I was working as Entomologist to the Sub-Committee on Midge Control, Scientific Advisory

Committee, Department of Health for Scotland. I should like to thank the members of the Sub-Committee for their constant support.

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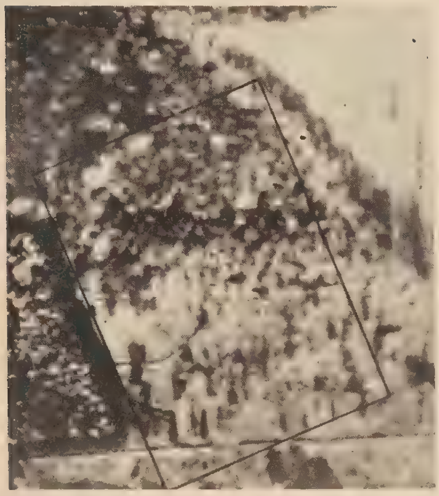

FIG. 1.

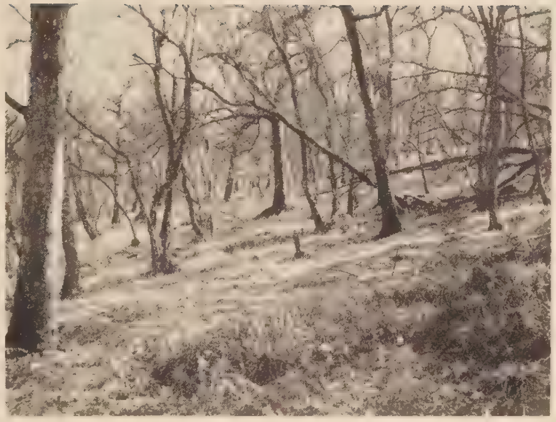

FIG. 3

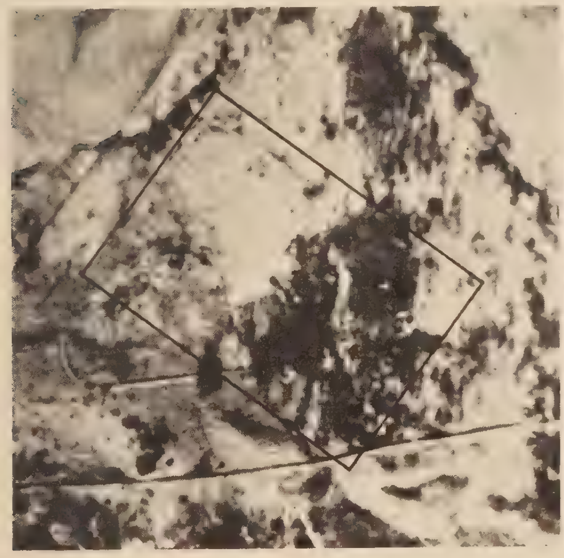

FIG. 2.

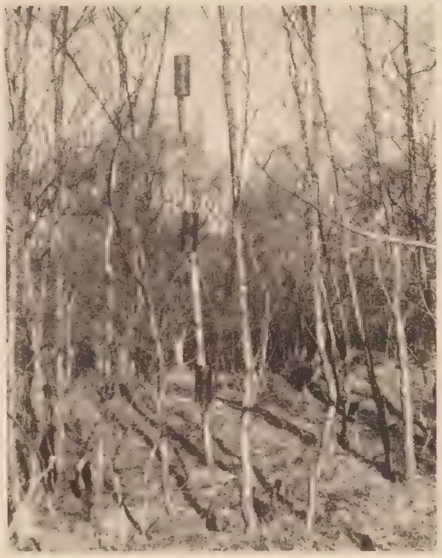

FIG. 4

FIG. 1. Aerial photograph of Bannachra, February, 1948. Rectangle indicates limits of experimental area. Scale approximately 16 inches to the mile. (Reproduced by kind permission of the Air Ministry, who retain the copyright.)

FIG. 2. Aerial photograph of Wester Bannachra. February, 1948. Rectangle indicates limits of experimental area. Scale approximately 16 inches to the mile. (Reproduced by kind permission of the Air Ministry, who retain the copyright.)

FIG. 3. View of a typical station in the oak wood in winter. This is station BW 11. (Reproduced by kind permission of the Editors of the *Annals of Tropical Medicine and Parasitology*.)

FIG. 4. View of a typical birch wood station in winter (Station BW 2).

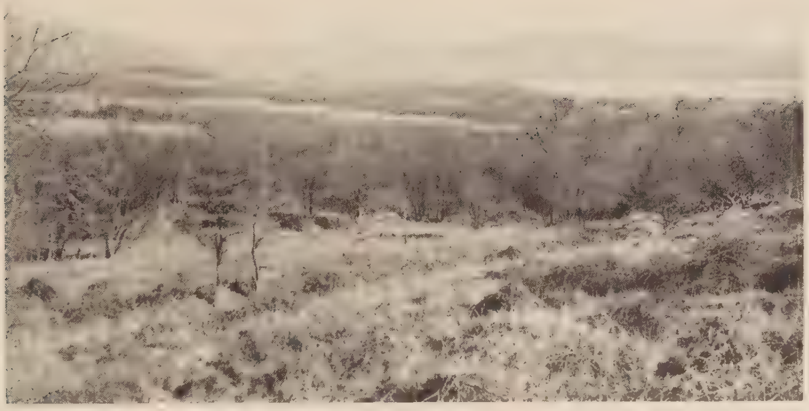

FIG. 1. View of Bannachra moorland in winter. Station BM 2 in the background. Note the abundance of dead bracken fronds. Loch Lomond can be seen in the distance.

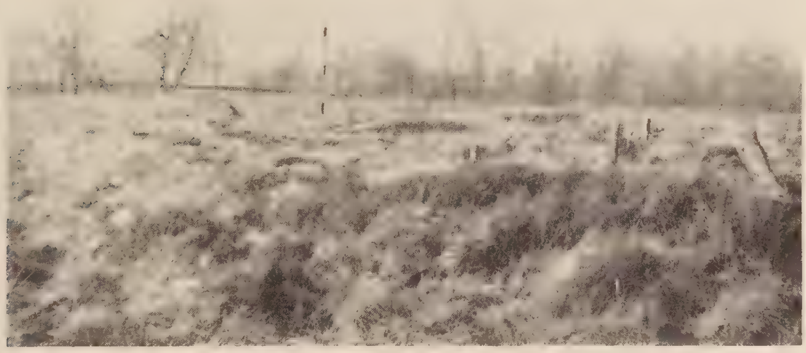

FIG. 2. View of Wester Bannachra moorland in winter. Stations WBM 5, WBM 7 and WBM 10 can be seen. The vegetation in the foreground is heather (*Calluna vulgaris*) and purple moor grass (*Molinia caerulea*).

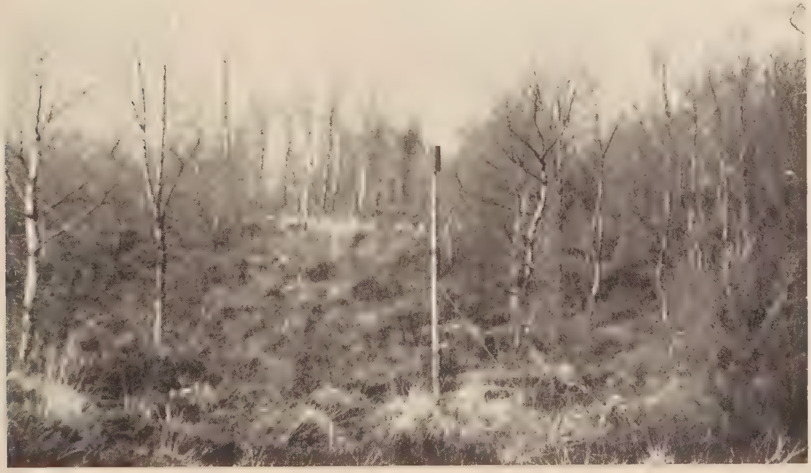

FIG. 3. View of a clearing in the wood at Wester Bannachra. Station WBW 5 in the foreground and WBW 2 in the background.

PIPERONYL BUTOXIDE AS A CONSTITUENT OF HEAVY-OIL SPRAYS FOR THE CONTROL OF STORED PRODUCT INSECTS.

I.—PIPERONYL BUTOXIDE AS A SYNERGIST FOR PYRETHRUM AND ITS EFFECT ON THE PERSISTENCE OF PYRETHRUM FILMS.

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The pyrethrins, in solution in Shell oil P31, are extensively used as sprays against stored product insects in this country. Their knock-down effect upon these insects is outstanding, and, because their toxicity to man is very slight, they can safely be sprayed on to foodstuffs ; but they are also expensive, and at economic concentrations are not very lethal to some stored product beetles. Possible substitutes such as DDT and BHC, which are among the safest of the newer synthetic insecticides, are on the whole more lethal to the insects, but remain restricted in use by their toxicity to man. However, it may be possible to improve upon the present sprays without substitutes. Certain substances, themselves but slightly toxic or non-toxic to insects, have been found to intensify the toxic action of the pyrethrins, and such synergists may enable the sprays to be formulated more economically. Sesamin, a constituent of sesame oil, is one of the best known of the synergists for pyrethrins, and research in the U.S.A. has related its synergistic properties to the methylene dioxyphenyl groups in the molecule. As a result compounds incorporating this group, including piperonyl butoxide and piperonyl cyclonene, are prepared commercially.

Piperonyl butoxide has been included in many pyrethrum preparations marketed in the U.S.A. Piperonyl butoxide appears to have been preferred to piperonyl cyclonene mainly because the former is fully soluble in oils and in the propellents in aerosol bombs, whereas the latter is not. Wachs (1947), however, implies that piperonyl butoxide is also the more active synergistically. Technical piperonyl butoxide is an amber-coloured liquid with a faint distinctive odour and a slightly bitter taste : it is described as stable (Dove, 1947). It contains 80 per cent. of (3, 4-methylene-dioxy-6-propyl benzyl) (butyl) diethylene glycol ether (Wachs, 1947). It is not yet available commercially in this country, but can be expected to become so before long. Technical piperonyl butoxide is referred to hereafter as PB.

PB alone has been found slightly, but definitely, toxic to house-flies (Wachs, 1947 ; Dove, 1947). However, its toxicity to mammals is exceedingly low (Sarles & others, 1949 ; Lehman, 1949). Lehman, in fact, describes combinations of pyrethrins and PB as among the safest insecticides from all standpoints. Pyrethrin-PB combinations should be as safe for direct application to foodstuffs as pyrethrins alone.

Pyrethrin-PB combinations have been tested in the U.S.A. and claimed effective against a wide variety of insects, including stored product pests (*cf.* Donohoe, 1948 ; Dove, 1947 ; Glass, 1948). However, the precise information available on the relative toxicities of different combinations is meagre, being almost confined to the papers of Dove (1947) and Wachs (1947). Mixtures of pyrethrins and PB have been incorporated in light-oil sprays, emulsions, aerosols, dusts, and wettable powders, and have been used for impregnating cotton bags (Cotton & others, 1949). Where pyrethrin-PB mixtures have replaced pyrethrins alone, it appears that the mixtures have been formulated so as to produce more effective but less costly preparations. Most of these formulations contain one part by weight of pyrethrins to 8–12 parts of PB, but the proportion is occasionally as low as 1 to 20 ; 1 to 10 is the most frequent proportion. Dove (1947) recommended a proportion of 1 to 8 for oil sprays for combating house-flies, but, although he published the data on which he based this recommendation, his reasons for choosing this proportion are not clear. Schroeder (1950) considered that varying the proportion between 1 to 2½ and 1 to 8 made little difference to the toxicity of aerosols if the concentrations were adjusted so as to keep the cost constant. Page & Blackith (1950) make the general, unqualified statement that raising the concentration of PB increases the toxicity of a mixture containing pyrethrins and PB only until a molecular ratio of 5 of the latter to 1 of the former is reached. However, the data of Dove (1947) and of Wachs (1947), together with those presented in this paper, show that the toxicity can increase for up to much higher molecular proportions.

Pyrethrin-PB combinations are claimed to produce highly persistent toxic residues on surfaces (Dove, 1947 : Donohoe, 1948), and it has therefore been suggested that PB exerts a stabilizing effect on the pyrethrins, as well as a synergistic effect, but Cotton & others (1948) found that PB failed to improve the persistence of films. The high boiling point of the major constituent of PB, 180°C. at 1 mm. pressure (see Wachs, 1947), indicates that the PB is sufficiently non-volatile to be lost only very slowly by evaporation from films.

PB has been shown in the U.S.A. to be a powerful synergist for pyrethrins. It has not been used there as a synergist of pyrethrins in P31, or comparable heavy oils, and an investigation was therefore started to assess the value of pyrethrin-PB combinations in P31 as sprays against stored product insects. The supposed stabilizing effect of PB on pyrethrins was considered of outstanding importance, as film effects are largely relied upon in this country in the control of stored product insects by sprays. For this reason, the effects of PB on film persistence were looked into before investigating the toxicities of different pyrethrin-PB formulations.

Materials and Methods.

The test insects were adult *Tribolium castaneum* (Hbst.), *Calandra granaria* (L.), *C. oryzae* (L.), and *Ptinus tectus* Boield. The *T. castaneum* were bred as described by Parkin and Green (1943), but at 25°C. instead of 24°C., and used when 3–5 weeks old. The beetles of the remaining species were taken from insectary cultures and were of unknown age. They were prepared for the tests in the same way as the *T. castaneum*, *i.e.*, counted into batches and left about 16 hours at 25°C. and 70 per cent. R.H. before exposure to the insecticides. The *P. tectus* were allowed to drink by confining them for the 16 hours before exposure with pieces of wet filter paper.

In the film tests, the insects were confined continuously on the films, usually for periods of six to nine days, on Whatman No. 544 filter papers or on wood samples. The wood was rough Western Red Cedar, and each sample measured about 3 ins. × 3 ins. × ½ in.

The solutions containing pyrethrins were derived from a concentrate in Shell oil P31 containing 11·74 per cent. w/v pyrethrin I and 8·76 per cent. w/v pyrethrin II, as determined by the Seil method. The concentrate was first diluted with P31 to make a stock solution, containing 5 per cent. total pyrethrins, which (like the concentrate) was stored in the dark at 0–2°C. Solutions for the experiments were prepared on the day of use by diluting the stock solution. The PB solutions were prepared by diluting technical piperonyl butoxide : the two samples used were shown to synergize equally. The concentrations of pyrethrins are expressed as per cent.

w/v, *i.e.*, g./100 ml. of solution ; but the concentrations of PB as per cent. v/v, *i.e.*, ml. of the technical material /100 ml. of solution.*

All the film tests were carried out at 25°C. and 70 per cent. R.H. Where the films were on filter papers, the technique of Parkin & Green (1943) was used. The deposits recorded in the tables refer to the weight of insecticide as a whole. The glass rings confining the *T. castaneum* were covered with wire gauze to prevent the beetles from flying out ; those confining the *C. granaria* were smeared internally with P31, but too lightly for the oil to have any toxic effect. Those confining the *C. oryzae* were covered with weighted glass plates ; and those confining the *P. tectus* were covered with bolting silk held in place by rubber bands. These modifications in technique were necessitated by the different behaviour of the test insects. The technique of spraying the wood samples and confining the *T. castaneum* on them is described by Parkin and Hewlett (1946). The direct spray technique is described by Hewlett (1947a) : the beetles were sprayed at 20–21°C. and 70 per cent. R.H. and afterwards kept at 25°C. and 70 per cent. R.H.

In order to assess the effects of the insecticides, the insects were examined under a low-power binocular microscope, and the numbers dead and knocked down determined as by Hewlett (1947a) and Parkin & Hewlett (1946). An insect was considered dead if it neither moved spontaneously, nor responded by reflex movement when touched with a brush. It was considered knocked down if, when not disturbed in any way, it lay on its back, unable to regain the normal position. Thus the insects counted as knocked down included those dead, and knock-down as defined did not necessarily occur shortly after the treatment with insecticide.

Preliminary Experiments.

In two preliminary experiments, *T. castaneum* were exposed to films and direct sprays of 1·6 per cent. pyrethrins, 1·6 per cent. pyrethrins+5 per cent. PB, and 5 per cent. PB. The results are shown in Tables I and II.

TABLE I.

The toxicity to *T. castaneum* of films on filter paper formed by pyrethrins, piperonyl butoxide, and the two together.

Insecticide	Number of beetles	Deposit mg./10 sq. cm.	Mortality per cent. after 6 days	Mortality per cent. after 9 days
1·6 per cent. pyrethrins	50	16·44	26	56
	51	18·41	27	63
	51	21·84	78	90
	50	23·40	86	98
1·6 per cent. pyrethrins + 5 per cent. PB	50	15·95	78	90
	50	18·85	86	98
	50	20·67	98	100
	49	23·43	100	100
5 per cent. PB	50	15·38	4	20
	52	18·30	8	31
	49	21·04	0	26
	50	24·44	4	20
Control	100	—	0	0

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* The difference between the specific gravities of P31 and PB (0·86 and 1·06 respectively) introduced a systematic error which was not allowed for. For a given deposit of insecticide as a whole, the quantity of PB applied was not exactly proportional to its concentration (per cent. v/v) in the solution. In the only experiment in which the error might have been of serious consequence (pp. 300–304), the maximum concentration of PB was 8 per cent., for which the deposit should really have been only 1·7 per cent. higher than that in fact aimed at.

Tables I and II show clearly that the PB greatly increased the toxicity of the pyrethrins. The 5 per cent. PB was virtually non-toxic as a direct spray, and as a film for six days. After nine days exposure of the insects, however, the films of 5 per cent. PB had produced an appreciable mortality, but this was apparently not related to the deposit.

The experiments indicated that PB was well worth further investigation with a view to its inclusion in pyrethrum-in-P31 sprays for use against stored product insects.

TABLE II.

The toxicity to *T. castaneum* of direct sprays of pyrethrins, piperonyl butoxide, and the two together, as shown by percentage mortality in batches of 46–52 beetles.

Days after spraying	Deposit mg. /10 sq. cm.	1·6 per cent. pyrethrins	1·6 per cent. pyrethrins +5 per cent. PB	5 per cent. PB	Control*
6	—				0
	2·00	9	86	0	
	3·17	28	98	0	
	5·04	55	92	0	
	8·00	74	98	0	
9	—				0·5
	2·00	15	92	2	
	3·17	38	100	0	
	5·04	63	100	2	
	8·00	86	100	2	

* 200 beetles.

The Toxicity of Piperonyl Butoxide alone.

In order to be able to interpret adequately experiments on the toxicity of pyrethrin-PB mixtures, it was necessary to know the toxicity of PB alone. Table III shows the toxicities of different concentrations of PB to *T. castaneum*. In view of the results of Tables I and II, the range of concentrations was made higher for the direct sprays than for the films.

TABLE III.

The toxicity to *T. castaneum* of piperonyl butoxide, applied as films on filter paper and as direct sprays : mortalities in batches of 50 beetles.

Method of application	PB per cent.	Deposit mg. /10 sq. cm.	Mortality per cent. after 6 days	9 days
Film	2·5	19·01	6	20
	5	19·40	0	12
	10	19·32	2	22
	20	18·21	8	26
Direct spray	5	8·00	0	2
	10	8·00	0	0
	20	8·00	2	6
	40	8·00	2	6
—	Control*	—	1	2·5

* 200 beetles.

Table III shows that as films the different concentrations of PB were barely toxic after six days exposure, but again appreciably toxic after nine days. The knock-down, which is not recorded, was only slightly higher than the mortality, and both were but slightly correlated with the PB concentration. The direct sprays of 8 mg. /10 sq. cm. of up to 40 per cent. PB were virtually non-toxic.

Table IV indicates the toxicity of films of 5–40 per cent. PB to *C. granaria, C. oryzae,* and *P. tectus.* The deposit was kept relatively low to avoid the *C. granaria* being killed by P31 (see Hewlett, 1947b).

TABLE IV.

The toxicity of piperonyl butoxide, as films on filter paper, to *C. granaria, C. oryzae,* and *P. tectus.* Exposure period : 6 days.

Species of beetle	Number of beetles	PB per cent.	Deposit mg. /10 sq. cm.	Knock-down per cent.	Mortality per cent.
C. granaria ...	50	5	15·32	20	12
	50	10	15·53	28	8
	49	20	15·45	30	16
	50	40	15·64	44	16
	100	Control	—	21	15
C. oryzae ...	50	5	15·90	22	10
	49	10	16·08	60	16
	50	20	16·10	90	52
	50	40	16·18	96	68
	100	Control	—	5	5
P. tectus ...	25	5	15·48	4	4
	25	10	15·32	4	0
	25	20	15·82	8	4
	25	40	16·49	0	0
	50	Control	—	0	0

The results with *Calandra* after nine days' exposure were not worth recording because of high control mortality. From Table IV it appears that *C. granaria* and *P. tectus* were resistant to PB, but that *C. oryzae* was somewhat susceptible. In this experiment the rings confining the *C. oryzae* were covered with glass plates, but a subsequent experiment designed to test for fumigant effect, that is not described here, showed an absence of any such effect.

The Effect of Piperonyl Butoxide on Film Persistence.

In examining the effect of PB on film persistence, a mixture of pyrethrins and PB, initially equal in toxicity to 1·3 per cent. pyrethrins, was first determined, and then the persistences of the mixture and of the pyrethrins alone were compared. A pyrethrin concentration and a pyrethrin-PB mixture of the same initial toxicity were prerequisite. It was clearly useless to compare the persistence of, say, 1·3 per cent. pyrethrins+5 per cent. PB with that of 1·3 per cent. pyrethrins alone, because the former would be more toxic initially than the latter, and so, having more toxicity to lose, could be expected to produce more persistent films, irrespective of whether the PB has some special stabilizing effect upon the pyrethrins.

Determination of a mixture of pyrethrins and piperonyl butoxide equal in toxicity to 1·3 per cent. pyrethrins.

The toxicity of 1·3 per cent. pyrethrins was compared with that of 0·65 per cent. pyrethrins combined with 0, 2·5, 5, 10, and 20 per cent. PB, exposing *T. castaneum* on filter paper. While 0·65 per cent. pyrethrins alone was considerably less lethal than 1·3 per cent., all the mixtures of 0·65 per cent. pyrethrins with PB were considerably more lethal than 1·3 per cent. pyrethrins. A similar test was therefore carried out to compare 1·3 per cent. pyrethrins alone with 0·1, 0·2, 0·4, and 0·65 per cent. pyrethrins each combined with 2·5 per cent. PB. The mixture containing 0·2 per cent. pyrethrins was definitely less and that containing 0·65 per cent. definitely more toxic than 1·3 per cent. pyrethrins alone : 0·4 per cent. pyrethrins+2·5 per cent. PB was probably about as toxic as 1·3 per cent. pyrethrins alone, but the results for this particular mixture were very heterogeneous. The test was therefore repeated and the results are given in the six left-hand columns of Table V, where the relevant mortalities appear under " 1st exposure ".

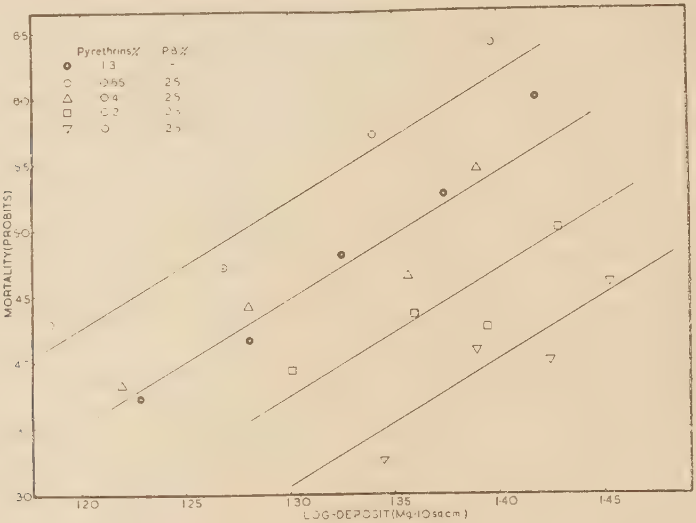

Fig.1.—The toxicity to *T. castaneum* of films formed by pyrethrin-PB mixtures and by 1·3 per cent. pyrethrins alone (6-day results).

Fig. 1 shows the 6-day results (1st exposure) of Table V : the lines were fitted by eye. Evidently 0·4 pyrethrins+2·5 per cent. PB was in fact about as lethal as 1·3 per cent. pyrethrins, and interpolation to obtain a more accurate estimate of the concentration of pyrethrins in the equivalent mixture was not worth while. The 9-day results were very similar.

It should be emphasised that 0·4 per cent. pyrethrins+2·5 per cent. PB and 1·3 per cent. pyrethrins were equally lethal. An adequate comparison of their knock-down effects was not obtained : at 6 days both gave 98–100 per cent. knock-down at all the doses used. The knock-down due to 0·2 per cent. pyrethrins+2·5 per cent. PB was, however, definitely lower (90–96 per cent.), and that due to 0·1 per cent. pyrethrins+2·5 per cent. PB lower still (64–86 per cent.).

The persistence of the selected mixture of pyrethrins and piperonyl butoxide.

In order to examine in a preliminary manner the effect of PB on film persistence, more *T. castaneum* were exposed on the filter papers used in the experiment just described. Thus, after the 9-day observations recorded in Table V under " 1st exposure " had been made, the beetles remaining on the filter papers were removed and fresh ones added. The mortality among these fresh beetles was determined, as usual, after 6 and 9 days exposure, that is when the films were 15 and 18 days old. The results are given in Table V, under " 2nd exposure ". The 6-day results of the second exposure are shown in fig. 2. Comparison of the corresponding mortalities

TABLE V.

The toxicity to *T. castaneum* of mixtures containing 2·5 per cent. piperonyl butoxide and different concentrations of pyrethrins, applied as films on filter papers.

Pyrethrins per cent.	PB per cent.	Deposit mg./10 sq. cm.	1st exposure (0–9 days)			2nd exposure (9–18 days)		
			Number of beetles	Mortality per cent. after 6 days	Mortality per cent. after 9 days	Number of beetles	Mortality per cent. after 6 days	Mortality per cent. after 9 days
1·30	—	16·91	50	10	42	50	10	24
		19·06	50	22	54	50	32	70
		21·14	50	42	74	32	12·5	46·9
		23·66	48	60·4	83·3	50	22	68
		26·21	50	84	98	50	34	64
0·10	2·5	22·15	50	4	26	50	6	20
		24·57	50	18	44	50	8	20
		26·60	50	16	44	49	22·4	67·3
		28·36	50	34	66	50	6	46
0·20	2·5	20·00	49	14·3	34·7	50	32	56
		22·91	50	26	52	49	6·1	38·8
		24·83	51	23·1	55	50	2	24
		26·88	50	50	80	49	16·3	51·0
0·40	2·5	16·55	50	12	40	50	4	30
		19·04	50	28	50	50	24	46
		22·73	50	36	70	50	26	44
		24·57	49	67·3	85·7	50	34	54
0·65	2·5	15·32	50	24	50	51	33·3	43·1
		18·55	49	38·8	67·3	30	36	54
		21·90	50	76	86	50	42	66
		24·94	50	92	100	50	56	78
Control		—	200	0·5	1·0	200	0·5	2·5

for the 1st and 2nd exposures shows that at higher deposits the films were considerably more toxic up to 9 days after deposition than 9–18 days after. The difference at lower deposits was less for the three most toxic insecticides, resulting in two regression lines of lower slope for the later exposure. Fig. 2 and the two right-hand columns of Table V indicate, despite considerable heterogeneity, that 0·4 per cent. pyrethrins+2·5 per cent. PB was about as lethal as 1·3 per cent. pyrethrins in the later exposure period, as in the earlier. The knock-down in the later exposure period was also about the same (90–100 per cent., varying according to dose) for this mixture as for the pyrethrins alone.

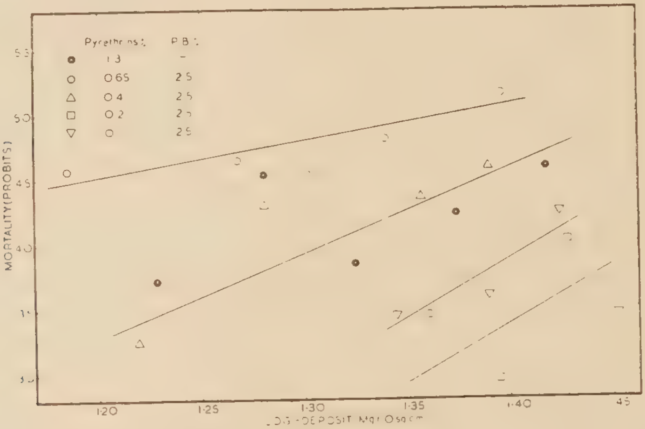

Fig. 2.—The toxicity to *T. castaneum* of films nine days old when the beetles were first exposed (6-day results).

The effect of PB on film persistence was further investigated by following the loss of toxicity of films on wood. One set of wood blocks was sprayed with 15 mg./10 sq. cm. of 0·4 per cent. pyrethrins+2·5 per cent. PB, and a duplicate set similarly sprayed with 1·3 per cent. pyrethrins alone. Beetles were exposed in batches of 50 at different times after spraying, two batches for each treatment and each exposure. Control batches were kept for each exposure period, and the control mortality never exceeded 4 per cent. The replication in knock-down and mortality among the similarly treated batches of insects was on the whole good, so most of the derived pairs of percentages were averaged in constructing Table VI.

The mortalities in Table VI indicate that the films formed by the mixture and the pyrethrins alone lost their toxicity at about the same rate up to and including the 11th day : the comparison at the 15th day is uncertain owing to bad replication for the mixture. The figures for knock-down forbid a direct comparison between the stabilities of the two types of film, since the pyrethrins alone produce a consistently higher knock-down than the mixture. However, if the films formed by the mixture had been markedly the more persistent, the knock-down they gave in the later exposure periods should have equalled or exceeded that for the films formed by the pyrethrins alone. It did not.

Thus the two experiments did not confirm claims that PB makes pyrethrum films more persistent. Though not exhaustive, they could hardly have failed to reveal any pronounced increase in persistence.

Pyrethrin Equivalents of Pyrethrin-Piperonyl Butoxide Mixtures.

An experiment was carried out to compare the toxicities of various concentrations of pyrethrins and pyrethrin-PB mixtures. It was evident from the previous

TABLE VI.

The persistence of films formed by 1·3 per cent. pyrethrins and 0·4 per cent. pyrethrins+2·5 per cent. piperonyl butoxide on wood, as shown by their toxicity to *T. castaneum.*

Film age in days when insects applied	Knock-down per cent. after				Mortality per cent. after			
	6 days		9 days		6 days		9 days	
	Pyrethrins alone	Pyrethrins +PB	Pyrethrins alone	Pyrethrins +PB	Pyrethrins alone	Pyrethrins +PB	Pyrethrins alone	Pyrethrins +PB
1	82	72	86	80	12	20	37	46
2	80	46	92	86	12	15	49	61
4	76	25	84	68	6	2	38	28
9	69	30	92	42	3	1	23	20
11	53	30	75	47	4	5	15	15
15	51	46,18*	59	50,24*	5	10,2*	35	52,10*

* Owing to bad replication, the percentages in each batch of 50 beetles is given.

experiments that the probit-log-deposit regression lines for pyrethrins alone and for the mixture were parallel ; indicating that, in its lethal film effects upon *T. castaneum*, a pyrethrin PB mixture could be regarded as a higher concentration of pyrethrins alone. This was the more reasonable as PB did not make the films more persistent. The object of the experiment was therefore to express the concentration of pyrethrins alone, Z, as a function of the pyrethrin and PB concentrations, z_1 and z_2 respectively, in a mixture of equal toxicity. This was done by finding

$$y=F(Z)$$
$$\text{and } y=f(z_1, z_2),$$

where y is mortality. Equating F(Z) and $f(z_1, z_2)$ then gave the required relationship. The best procedure is to represent all the data, including those for pyrethrins alone, by a single function $y=f(z_1, z_2)$ and then to obtain $y=F(Z)$ by replacing z_1 with Z in $f(z_1, 0)$. The single function, however, must then be of suitable form, and a suitable function was not found.

In the experiment, batches of 49–50 *T. castaneum* beetles were exposed in duplicate to Whatman No. 544 filter papers sprayed with 0·650, 0·919, 1·30, and 1·84 per cent. pyrethrins alone ; and to each combination of 0·081, 0·162, 0·325, and 0·650 per cent. pyrethrins with 1, 2, 4, and 8 per cent. PB. The deposit was about 23 mg./10 sq. cm. (22·39–23·69 mg./10 sq. cm. ; but 34 of the 40 deposits were in the range 22·47–23·51 mg./10 sq. cm.).

On the whole the agreement within the pairs of batches similarly treated was good. The high mortalities shown by one replicate of 0·919 per cent. pyrethrins were judged, on the basis of an immediately previous experiment, to be exceptionally high and were therefore ignored in analyzing the results. Table VII gives the mean mortalities for the different insecticidal solutions. The control mortalities (in 200 beetles) were zero and 0·5 per cent. at six and nine days respectively.

The results for six days were, as usual, clearer than those for nine days, and so the former were examined in more detail. The six-day mortalities, expressed as probits, for pyrethrins alone were found to be linearly related to log-concentration within the range examined, and the regression equation was computed as

$$Y = 4{\cdot}617 + 4{\cdot}152 \log Z. \qquad (1)$$

For this equation $\chi^2=2{\cdot}78$ on 2 degrees of freedom, whence $P=0{\cdot}24$.

TABLE VII.

The percentage mortalities (each based on 99–100 beetles) of *T. castaneum* produced by pyrethrins, alone or combined with piperonyl butoxide after six days (after nine days in brackets).

Pyrethrins per cent.	PB %				
	0	1	2	4	8
0·081		12 (39)	13 (45)	12·1 (47·5)	13·1 (34·4)
0·162		7 (25)	9 (31)	23 (46)	30 (44)
0·325		23 (38)	43 (58)	66 (77)	80 (96)
0·650	12 (38)	32* (60*)	80 (92)	94 (99)	97 (100)
0·919	36* (62*)				
1·30	47·5 (71·7)				
1·84	79 (95)				

*50 beetles.

Finding a relationship between mortality and the concentrations of pyrethrins and PB in the mixtures was more difficult. For the six-day and nine-day results taken together, reducing the pyrethrin concentration from 0·162 per cent. to 0·081 per cent. failed in the majority of cases to reduce the mortality. This was attributed to failure of this type of technique at low concentrations, comparable to its frequent failure at low deposits, and the results for 0·081 per cent. pyrethrins were therefore disregarded.

Graphical analysis of the remaining results indicated that probit mortality was separately linear in log-concentration of pyrethrins and log-concentration of PB, but not linear in either concentration untransformed. The most general expression linear in $\log z_1$ and $\log z_2$ is

$$Y=a+b_1 \log z_1+b_2 \log z_2+b_{12} \log z_1 \log z_2. \tag{2}$$

Furthermore, the graphical analysis indicated that the data could not adequately be represented by an expression simpler than (2), derived by putting b_1, b_2, or b_{12} equal to zero. Other types of relationship were tried without success. Equation (2) was then fitted to the data as described by Finney (1947), and one cycle of computations gave

$$Y=5{\cdot}271+2{\cdot}142 \log z_1+3{\cdot}113 \log z_2+2{\cdot}668 \log z_1 \log z_2. \tag{3}$$

The representation of the data by this expression is shown in figs. 3 and 4. Fig. 3 shows Y plotted against ($\log z_1+\log z_2$), and the points fitted to the two sets of diverging straight lines representing (3) : obviously the data would not fit a probit plane.

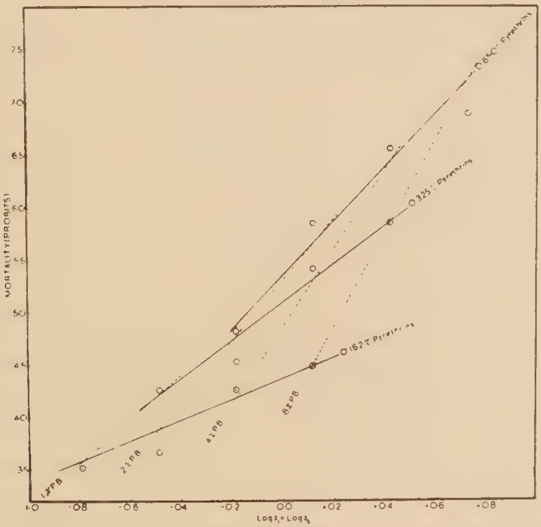

Fig. 3.—The toxicity to *T. castaneum* of films formed by different pyrethrin-PB mixtures (6-day results). Probit mortality is plotted against ($\log z_1+\log z_2$), where z_1 is pyrethrin concentration and z_2 is PB concentration. Obviously the data do not fit a probit plane.

The fit of (3) to the data may, however, be more easily appreciated in fig. 4. Here the observed probit mortalities have each been reduced by $2{\cdot}668 \log z_1 \log z_2$, and the reduced probits plotted against ($3 \log z_1+\log z_2$). The reduced probits are fitted by the first three terms of equation (3), *i.e.*, by a plane in $\log z_1$ and $\log z_2$, and when plotted in this way are represented by two sets of parallel straight lines. Normally (see Finney, 1947) probits would be plotted against ($\log z_1+\log z_2$) but the coefficients of log z_1 and log z_2 in (3) are not very different, and plotting instead against (3 log z_1 +log z_2) increases conveniently the difference between the slopes of the two sets of lines in the figure.

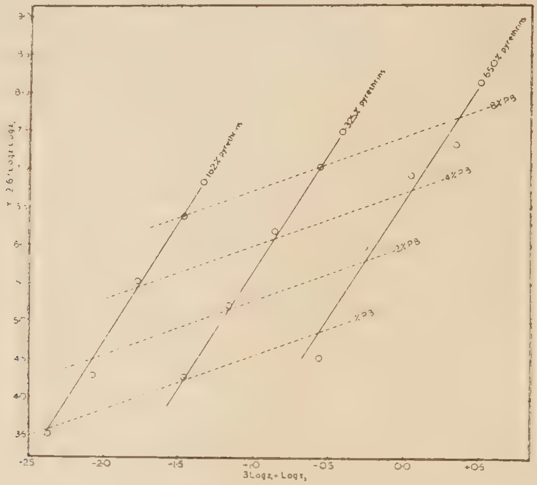

Fig. 4.—The toxicity to *T. castaneum* of films formed by different pyrethrin-PB mixtures (6-day results). Probits, reduced by 2·67 log z_1 log z_2, are plotted against (3 log z_1+log z_2), showing how the reduced probits can be represented by a plane.

A direct comparison (see Finney, 1947, p. 60) of the mortalities calculated from equation (3) with those observed yielded a χ^2 of 7·8 on 7 degrees of freedom, when P=0·35. Thus the observed and calculated mortalities agreed within the limits of sampling error. However part of the χ^2 is attributable to variation in deposit, a point discussed later (see p. 307).

Equating the right-hand sides of equations (1) and (3) gives

$$4{\cdot}617+4{\cdot}152 \log Z=5{\cdot}271+2{\cdot}142 \log z_1+3{\cdot}113 \log z_2+2{\cdot}668 \log z_1 \log z_2,$$

whence,

$$\log Z=0{\cdot}1575+0{\cdot}516 \log z_1+0{\cdot}750 \log z_2+0{\cdot}643 \log z_1 \log z_2. \qquad (4)$$

(4) Gives approximately the pyrethrin concentration, Z per cent. w/v, equal in lethal effect to z_2 per cent. w/v of pyrethrins combined with z_2 per cent. v/v of PB. Values of Z found by means of (4) are only approximate because the parameters of (1) and (3) are subject to error. Moreover (4) is valid only over the ranges of concentration used to determine it, that is, say, for Z=0·65 to 1·9 ; z_1=0·15 to 0·65 ; z_2=1 to 8. It should not be used for values outside these limits.

The pyrethrins equivalent of 0·4 per cent. pyrethrins+2·5 per cent. PB was calculated by means of the formula (4), which gave Z=1·41 ; *i.e.* 1·4 per cent. pyrethrins as a film on filter paper is about as lethal to *T. castaneum* beetles as 0·4 per cent. pyrethrins+2·5 per cent. PB. This agrees well with the figure of 1·3 per cent. by the earlier direct, and probably less accurate, determination (see p. 298).

The Influence of Species and Method of Application on Pyrethrin Equivalents.

In the experiment just described *T. castaneum* was used as the test insect, and the insecticidal solutions were applied as films. It was not known whether equation (4) would hold for a different species or a different method of application of insecticide, so experiments were carried out to discover this.

Influence of insect species.

In the direct determinations, as a film 0·4 per cent. pyrethrins + 2·5 per cent. PB was found to be about as lethal as 1·3 per cent. pyrethrins to *T. castaneum*. A comparison of the toxicity to *C. granaria* of these two solutions, similarly applied as films, indicated that the mixture was more toxic than the pyrethrins alone. The mortalities produced by 0·65 per cent. pyrethrins were markedly lower than those produced by 1·3 per cent., indicating that the technique was reliable when *C. granaria* was the test insect. When equation (4) had been computed, the pyrethrin equivalent for *T. castaneum* of the pyrethrin-PB mixture was found to be 1·4 per cent., so in a confirmatory test 0·4 per cent. pyrethrins + 2·5 per cent. PB was compared with 1·4 per cent. pyrethrins alone using *C. granaria*. The deposit was kept sufficiently low for the P31 to be non-toxic to the *C. granaria* (see Hewlett, 1947b). Table VIII gives the results.

TABLE VIII.

The toxicity to *C. granaria* of 1·4 per cent. pyrethrins alone and of 0·4 per cent. pyrethrins + 2·5 per cent. piperonyl butoxide, applied as films on filter paper.

Insecticide	Number of beetles	Deposit mg./10 sq. cm.	Mortality % after		
			3 days	4 days	5 days
1·4% pyrethrins ...	50	11·92	6	10	18
	50	12·39	0	8	20
	50	16·08	4	20	32
	50	16·44	6	14	26
0·4% pyrethrins + 2·5% PB	50	11·77	10	22	36
	50	12·52	16	30	44
	50	16·00	28	52	68
	50	16·21	20	42	58
Control	100	—	0	1	9

TABLE IX.

The toxicity to *T. castaneum* of 1·3 per cent. pyrethrins alone and combinations of 2·5 per cent. piperonyl butoxide with different concentrations of pyrethrins, applied as direct sprays.

Pyrethrins %	PB %	Number of beetles	Deposit mg./10 sq. cm.	Mortality % after	
				6 days	9 days
1·30	—	50	2·00	6	12
		50	2·99	26	44
		50	4·47	46	58
		47	6·69	78·7	83·0
		50	10·00	92	96
0·40	2·5	50	3·00	0	14
		50	4·76	10	24
		50	7·56	8	28
		50	12·00	22	48
0·65	2·5	50	2·00	18	40
		50	3·17	30	50
		50	5·04	56	68
		50	8·00	76	84
1·06	2·5	50	2·00	60	68
		50	2·99	74	80
		50	4·47	100	100
		50	6·69	94	96
Control	—	200	—	0·0	0·5

Table VIII shows that the mixture was decidedly more toxic than the 1·4 per cent. pyrethrins.

Influence of the method of application.

On comparing 0·4 per cent. pyrethrins+2·5 per cent. PB with 1·3 per cent. pyrethrins as direct sprays against *T. castaneum*, the former was found to be much the less toxic. In a further experiment 1·3 per cent. pyrethrins alone was compared with 0·40, 0·65, and 1·06 per cent. pyrethrins, each combined with 2·5 per cent. PB. The results are given in Table IX.

The six-day results are shown in fig. 5. It is obvious from Table IX and fig. 5 that 0·4 per cent. pyrethrins+2·5 per cent. PB was considerably less toxic as a direct spray than 1·3 per cent. pyrethrins alone, confirming the previous experiment. Furthermore, 0·65 per cent. pyrethrins+2·5 per cent. PB was about as toxic, and 1·06 per cent. pyrethrins+2·5 per cent. PB was considerably more toxic than 1·3 per cent. pyrethrins.

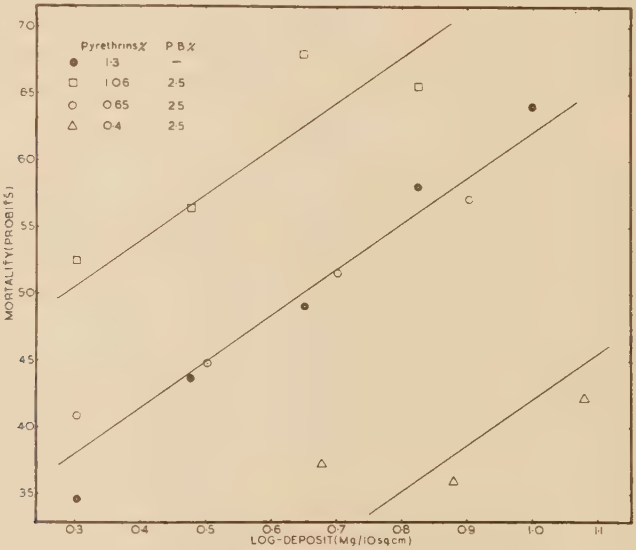

Fig. 5.—The toxicity to *T. castaneum* of direct sprays of pyrethrin-PB mixtures and of 1·3 per cent. pyrethrins alone (6-day results).

From these experiments it was concluded that the pyrethrin equivalent of a pyrethrin-PB mixture could differ according to the species of insect and method of application. Equation (4), unfortunately, can therefore be assumed to hold only for *T. castaneum* beetles treated by the film method.

Discussion.

Very little has been published concerning the mode of synergistic action of PB. Its synergistic action is not, however, confined to pyrethrins, for Brannon (1947) found that it synergized rotenone also. Moreover, tests by Piquett (1949) and Moore (1950) indicated that, as might be expected, it synergized a synthetic pyrethroid (the completely synthetic allyl homologue of cinerin I). However, PB has also been described as comparatively ineffective as a synergist for this synthetic

(Anon., 1950) : perhaps the synergistic effect varies considerably from one species of insect to another. Wilson (1949) found that PB synergized pyrethrins almost as well when the PB and the pyrethrins were applied respectively to different parts of individual house-flies as when they were applied together to the same part. This indicates that PB does not assist the pyrethrins to penetrate the insect cuticle. Wilson suggests that PB damages a mechanism in the insect by which the pyrethrins are normally detoxicated.

The work described in this paper was not concerned with the mode of action of PB. However, it was observed that the lethal action on *T. castaneum* of the films of PB alone was most unusually delayed. The PB had killed hardly a beetle after six days, but an appreciable percentage—20 per cent. or 30 per cent.—after nine days. In view of this increase in mortality between six and nine days, a given pyrethrin-PB mixture might have been expected to have been more toxic, relative to pyrethrins alone, at nine days than at six days ; but it did not appear to be so. This is readily explained, however, by assuming that (1) PB and pyrethrins have independent lethal actions, (2) the lethal and synergistic actions of PB are independent, and (3) the individual resistances to the lethal action of PB on the one hand and to that of pyrethrins or pyrethrins synergized by PB on the other, are strongly and positively correlated. On these assumptions the lethal action of PB would scarcely augment that of pyrethrins synergized by PB (see Plackett & Hewlett, 1948).

The method used for investigating the effect of PB on film persistence might appear open to objection. It might be argued that 0·4 per cent. pyrethrins+2·5 per cent. PB appeared approximately equivalent to 1·3 per cent. pyrethrins largely because PB increased the stability of the pyrethrins. However, if this were so, films more than nine days old formed by the mixture should have been more toxic than those formed by the pyrethrins alone. In view of the considerable comment on the supposed stabilization of pyrethrins by PB, it may seem surprising that the experiments reported here failed to reveal any such effect. On the other hand, the published evidence for the effect is far from conclusive.

Equation (2) was not an ideal relationship for fitting to the data from the experiment on the pyrethrin equivalents of pyrethrin-PB mixtures, as it gives absurd results on extrapolation. Thus, putting $z_2=0$ gives $Y=-\infty$, *i.e.* a percentage mortality of zero. This implies that, in the absence of PB, pyrethrins, in however large a dose, kill no insects. For this reason equation (4), derived by fitting (2), should be used only for the range of concentrations for which it was determined. Not only does the form of equation (2) limit its scope, but the statistical methods of fitting both (1) and (2) were not the most exact available. Ideally the systematic error mentioned on p. 295 should have been allowed for, and the effects of the variation in deposit separated out by covariance analysis (see Finney, 1947). However, these refinements would affect the final result but little.

The form of equation (4) precludes any very simple general statement of the pyrethrin equivalents of pyrethrin-PB mixtures. It cannot, for example, be stated that a mixture containing pyrethrins and PB in a given proportion is equivalent to pyrethrins alone at so many times the concentration of pyrethrins in the mixture.

The pyrethrin equivalent of a pyrethrin-PB mixture was shown to vary according to the method of application and the insect species. Further information on such pyrethrin equivalents is therefore desirable, especially for different species, so work on this point has been started. Although equation (4) may apply only to the particular conditions under which it was determined, it is instructive to consider some formulations based upon it. On the basis of (4), the following mixtures are all equivalent to 1·3 per cent. pyrethrins :—

Pyrethrins per cent. w/v	0·82	0·42	0·28	0·22
PB per cent. v/v ...	1	2	4	8

The lower the pyrethrin concentration the higher, of course, the PB concentration. In the absence of any special stabilizing effect, the cheapest formulation would be selected for use from the range of equivalent formulations. The relative prices of different formulations were calculated, assuming that pyrethrins are 12·3 times as expensive (weight for weight) as PB, and that P31 contributes 5 per cent. to the total cost of the spray. The assumptions appear reasonable in view of the prices now reigning. The calculations showed that a spray containing 0·35 per cent. pyrethrins+2·7 per cent. PB would be the cheapest substitute for 1·3 per cent. pyrethrins alone, the former costing only about 45 per cent. of the latter. However, it should be possible to go further. It should be possible to formulate a spray considerably more potent, but still much cheaper, than 1·3 per cent. pyrethrins alone in P31. Thus it has been calculated that the cheapest substitute for 1·8 per cent. pyrethrins would be 0·45 per cent. pyrethrins+3·5 per cent. PB, and that the cost of the latter in P31 would be only about 60 per cent. of that of 1·3 per cent. pyrethrins. Incidentally such a spray could be expected to form more persistent films, simply by virtue of their higher initial toxicity, than would 1·3 per cent. pyrethrins. The formulations and prices just quoted are, it should be emphasized, tentative ; but even if the figures of 45 per cent. and 60 per cent. should prove somewhat low, the prospects for pyrethrin-PB formulations, in place of pyrethrins alone, would remain most encouraging.

Summary.

Laboratory experiments showed that piperonyl butoxide (PB) is a powerful synergist of pyrethrins in Shell oil P31.

The toxicity of PB alone to different species of insects was determined. *Tribolium castaneum* was resistant to direct sprays of PB in P31, but appreciably susceptible to films : the toxic action of the films on this species was unusually delayed. *Ptinus tectus* and *Calandra granaria* were resistant to films, but *C. oryzae* somewhat susceptible.

In view of claims that PB makes pyrethrum films more persistent, an experiment specifically designed to reveal such an effect was carried out. This failed to show that PB makes the films any more persistent.

A formula was obtained giving the approximate pyrethrin equivalent of different pyrethrin-PB mixtures applied to *T. castaneum* as films on filter paper. It was shown, however, that the pyrethrin equivalent of a given pyrethrin-PB mixture could differ according to the method of application and according to the species of test insect.

Acknowledgements.

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FOOD-PLANTS OF CITRUS LEAF-MINER (*PHYLLOCNISTIS CITRELLA* STN.) IN THE PUNJAB.

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The Citrus Leaf-miner (*Phyllocnistis citrella* Stn.) has been recorded on a large variety of food-plants belonging to the families Rutaceae, Oleaceae, Loranthaceae, Lauraceae, and Leguminosae, which have no close relationship with each other and this naturally leads one to doubt the authenticity of some of the observations on record.

Stainton (1856) was the first to record it on *Citrus* sp. in India ; Sasscer (1915) recorded it from the United States on nursery stock of *Citrus* and *Atalantia* imported from the Philippine Islands ; Fletcher (1920) on Bael (*Aegle marmelos*) and Bela (*Jasminum sambac*) in India ; Reinking and Groff (1921) on *Loranthus* in the Philippine Islands ; Mitra and Khongwir (1928) on orange in Assam ; Clausen (1933) on *Citrus nobilis* and *Poncirus trifoliata* in Japan ; Margabandhu (1933) on *Murraya koenigii* in India and on *Pongamia glabra* and *Jasminum* in Northern Circars ; Pruthi and Mani (1945) on orange, pomelo, lemon, *Murraya exotica*, *M. koenigii*, willow, *Jasminum cinnamonum*, cinnamon, and *Alseodaphne semecarpifolia* in India.

The adults of *Phyllocnistis citrella* may alight on any plant and it appears that some of the food-plants have been recorded only because the adults were seen on them, irrespective of whether the pest is able to breed and feed on them. In view of the importance of citrus cultivation in the Punjab, it was considered necessary to have complete and accurate information of the alternative food-plants if the pest is to be controlled economically.

Food-plant Survey.

In order to ascertain the food-plants of *Phyllocnistis citrella*, a thorough search was carried out at Lyallpur and other localities in the Province. It was found to feed and develop through all its stages on Bael (*Aegle marmelos*) and the following species of citrus :—

Local name	*English name*	*Botanical name*
Khatti	Rough lemon	*Citrus limonia* Osbeck.
Khatta	Sour orange	*Citrus aurantium* Linn.
Malta	Sweet orange	*Citrus sinensis* Osbeck.
Sangtra	Mandarine orange	*Citrus nobilis* Lour.
Mitha	Sweet lime	*Citrus limetta* Risso
Kaghzi lime	Lime	*Citrus aurantiifolia* (Christm.).
Walayate nimboo	Lemon	*Citrus limonia* Osbeck
Galgal	Elephant lemon	*Citrus medica* Linn.
Chakotra	Pumelo, Shaddock	*Citrus maxima* Merill
Grape fruit	Grape fruit, Pomelo	*Citrus paradisi* Macf.

The food-plants recorded by previous workers were studied, and some others, to ascertain whether oviposition took place on these plants and whether, when oviposition occurred, it was followed by development through the larval and pupal stages.

Freshly sprouting leaves of the plants were enclosed in voile cloth sleeves for four to ten days before the liberation of adults. This was to afford time for any eggs, already laid, to hatch out. Only leaves found free from eggs were used. Five pairs of adults collected from the field or bred in the laboratory were introduced into each sleeve for 24–72 hours and then removed and liberated on to another shoot. This experiment was repeated twice in October and November. Oviposition was recorded by counting the number of eggs laid, and by counting the number of mines that appeared subsequently on each shoot. During the course of these studies oviposition by ten pairs and the development of other stages were studied on each plant. The results are recorded in Table I.

TABLE I.

Oviposition and development of pre-imaginal stages on various plants.

	No. of eggs laid by 10 pairs	No. of mines formed	No. developed into pupae	No. of adults emerged from pupae
Eugenia jambolana	0	0	0	0
Grewia asiatica	3	0	0	0
Aegle marmelos	11	8	6	6
Willow (*Salix* sp.)	4	1	0	0
Grapevine (*Vitis vinifera*) ...	4	0	0	0
Murraya exotica	5	2	0	0
Murraya koenigii	4	0	0	0
Dalbergia sissoo	5	0	0	0
Jasminum sp....	2	0	0	0
Jasminum sambac	2	0	0	0
Peas (*Pisum sativum*) ...	0	0	0	0
Convolvulus arvensis	0	0	0	0
Cichorium intybus	0	0	0	0
Malva parviflora	0	0	0	0
Lamnea nudicalus	0	0	0	0
Verbena (*Verbena* sp.) ...	0	0	0	0
Loranthus (*Loranthus* sp.) ...	0	0	0	0

It is seen from the above Table that the plants fall into four categories :—

(1) Those on which no oviposition took place : *Eugenia jambolana, Cichorium intybus, Malva parviflora,* Verbena (*Verbena* sp.), *Lamnea nudicalus,* peas (*Pisum sativum*), *Convolvulus arvensis* and Loranthus (*Loranthus* sp.).

(2) Plants on which a limited amount of oviposition took place but which were unsuitable for pre-imaginal stages : grapevine (*Vitis vinifera*), *Jasminum sambac, Jasminum* sp., *Dalbergia sissoo, Murraya koenigii* and *Grewia asiatica.*

(3) Plants suitable for oviposition but only partially so for pre-imaginal stages : willow (*Salix* sp.) and *Murraya exotica.*

(4) Plants suitable for complete development, *Aegle marmelos.*

Host Preference.

Little work has been carried out to determine the susceptibility of different food-plants to the attack of *P. citrella.* Observations to establish the susceptibility of various plants of economic importance were, therefore, recorded over a period of two consecutive years.

Since the pest invariably infests fresh top and terminal foliage, the fresh growth every month was marked by tags. The total number of fresh leaves produced by the plant was recorded and the number of these attacked by the pest counted every month. The observations were recorded in terms of percentage of attack. The results are presented in Table II.

TABLE II.

Percentage of attack on top fresh leaves of various plants.

Plants	July	August	September	October	November	December	January	February	March	April	May	June
Sour orange (*Citrus aurantium*)	24	28·5	49·5	78	78	34·5	28	27	28	33·5	17	26
Sweet lime (*C. limetta*) ...	51·5	53	52·5	72·5	73·5	60	43	34	28·5	20·5	22·5	39
Mandarine orange (*C. nobilis*) ...	20·5	23·5	53·5	41·5	60	35	29	22	19·5	34·5	25·5	22
Lime (*C. aurantiifolia*)	24·5	28·5	53	40	46	26	25	19	21·5	21·5	22·5	27
Elephant lemon (*C. medica*) ...	40	44	47	100	100	100	22	18	34	36·5	42	46
Pumelo, Shaddock (*C. maxima*)	12	17	27·5	67	57	53	19	22	21·5	22	29·5	30
Sweet orange (*C. sinensis*) ...	43·5	51·5	59·5	100	91	73·5	25	25	28·5	30	25·5	34
Bael (*Aegle marmelos*)	1·2	—	—	—	—	—	—	—	—	1·25	0·75	0·75

It will be noticed from Table II that the attack on *Aegle marmelos* is almost negligible, whereas it is severe on all the species of *Citrus*. *Citrus medica* is the most severely attacked while *C. maxima, C. nobilis* and *C. aurantiifolia* are the least heavily infested.

The existence of host preference for oviposition was also studied in detail. For this purpose 40 infested leaves of each of the food-plants were collected at random and examined carefully for the presence of eggs and mines. It was observed that while leaves of *C. aurantiifolia* had, on an average, two mines and eggs per leaf, *C. medica* and *C. maxima* possessed, on an average, 4·3 and 4·2 mines and eggs per leaf, respectively. The maximum number of eggs and mines was also the highest in case of *C. medica*, being 21 per single leaf. It was least in the case of *C. aurantiifolia*, European lemon (*C. limonia*) and *Aegle marmelos*, where the maximum number of eggs and mines was seven only.

The data collected were analysed statistically, and the results of analysis of variance are presented in Table III.

TABLE III.

Analysis of variance.

	Degrees of freedom (D.F.)	Sum of squares (S.S.)	Mean square (M.S.)	Variance ratio (F.)
Observations	39	459·3	11·0	3·0
Plants	10	211·8	21·2	
Error	390	2,731·8	7·0	
Total	439	3,402·9		

Standard error (S.E.) =0·42.
Critical difference at 5 per cent. = 1·2.
Critical difference at 1 per cent. = 1·5.

Order of Infestation.

Elephant lemon (*C. medica*)	4·3
Grapefruit, Pomelo (*C. paradisi*)	4·2
Rough lemon (*C. limonia*)	4·0
Pumelo, Shaddock (*C. maxima*)	4·0
Sour orange (*C. aurantium*)	3·8
Sweet lime (*C. limetta*)	3·3
Sweet orange (*C. sinensis*)	3·0
Bael (*Aegle marmelos*)	3·0
Lemon (*C. limonia*)	2·8
Mandarine orange (*C. nobilis*)	2·6
Lime (*C. aurantiifolia*)	2·0

The results are highly significant. The infestation in the form of eggs and mines is the highest in *C. medica* and the least in *C. aurantiifolia*.

The length of the mines on different food-plants varies considerably. This fact was studied critically to see whether mine length could form an index to host preference. For this purpose 50 mines were selected at random from infested leaves of each of the food-plants and measured by means of a divider with its points half a centimeter apart. The record of these measurements is given in Table IV.

TABLE IV.

Maximum, minimum and average length of mines in different food-plants.

Plant	Maximum cms.	Minimum cms.	Average cms.
Elephant lemon (*C. medica*)	30·0	14·8	24·5
Bael (*Aegle marmelos*)	24·0	15·5	21·8
Pumelo, Shaddock (*C. maxima*) ...	28·0	14·7	21·5
Grapefruit, Pomelo (*C. paradisi*) ...	26·5	16·2	21·4
Rough lemon (*C. limonia*)	25·0	15·0	21·2
Lemon (*C. limonia*)	24·0	14·7	21·1
Mandarine orange (*C. nobilis*) ...	26·2	15·4	18·0
Sweet lime (*C. limetta*)	21·5	12·5	18·0
Sour orange (*C. aurantium*)	21·5	15·0	17·9
Sweet orange (*C. sinensis*)	23·5	12·2	17·8
Lime (*C. aurantiifolia*)	17·2	12·2	16·5

TABLE V.

Analysis of variance.

	D.F.	S.S.	M.S.	F.
Observations	49	889·51	12·03	38·6
Plants	10	2,980·71	298·07	
Error	490	3,781·49	7·71	
Total	549	7,351·71		

S.E. ... ... =0·39.
Critical difference at 5 per cent.=1·078.
Critical difference at 1 per cent.=1·416.

It will be seen from Table IV that *C. medica* is the most favoured food-plant, having the highest maximum and average length of mines, and that *C. aurantiifolia* is the least preferred. The results also are shown to be highly significant from the analysis of variance given in Table V. This preference of food-plant is due to the nature of the parenchymatous cells and the epidermal layer ; plants having succulent leaves with thin-walled parenchymatous cells and a thin cuticle provide a more suitable medium for the mining activities of the larvae. An examination of the cross section of leaves shows that those of *C. aurantiifolia* are comparatively thicker and coarser than those of *C. medica* and of the other plants listed in Table IV.

Summary.

The Citrus Leaf-miner (*Phyllocnistis citrella*) has been recorded from plants belonging to several widely differing botanical families.

It is shown that the true food-plants of this pest are the various species of *Citrus* and *Aegle marmelos.*

Elephant lemon (*Citrus medica*), which has comparatively succulent leaves, is the most susceptible species of *Citrus*, whilst lime (*C. aurantiifolia*), with its comparatively thick and coarse leaves, is the least susceptible. *Aegle marmelos* is not of any importance as the attack in this case is negligible.

C. medica should be excluded from citrus orchards as it is of least economic importance, and is highly susceptible to attack. *C. limonia*, which is usually grown as a hedge around gardens, provides an undisturbed breeding ground for the pest and should as far as possible not be planted around citrus plantations.

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PROBABLE INSECT VECTORS OF YELLOW FEVER VIRUS, FROM MONKEY TO MAN, IN BWAMBA COUNTY, UGANDA.

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It has been known for several years that immunity to yellow fever virus is widespread among the monkeys of uninhabited forest in Central Africa (Haddow, Smithburn, Mahaffy & Bugher, 1947). Considerable evidence has been adduced, particularly from Bwamba, Uganda, to show that virus is disseminated among the monkey population by *Aëdes* (*Stegomyia*) *africanus* (Theo.) (Haddow & others, 1948 ; Smithburn & others, 1949). *A.* (*S.*) *aegypti* (L.), although not uncommon as a larva in Bwamba, particularly in tree holes, is very rarely taken biting (Haddow, 1945a). It is considered to have little or no importance as a vector of yellow fever in this locality ; in the human population the principal vector appeared to be *A.* (*S.*) *simpsoni* (Theo.) (Mahaffy & others, 1942). Smithburn and Haddow (1946) record the isolation of yellow fever virus from *A. simpsoni* in a forest-edge plantation eleven months after the entire human population had been immunised by means of vaccination which tended to confirm the suggestion that this mosquito acquired its infection from forest animals. The method of transference of virus from the monkey to the human population has not, however, been closely examined in relation to the habits of the natives, and the present work is an attempt to throw more light on that matter. G. W. A. Dick (unpublished, 1948) has shown that, in 1946 and 1947, 590 Bwamba children aged one to four years, who were unborn at the time of the vaccination campaign in 1941, were 39·8 per cent. immune to yellow fever. The virtually complete immunisation in 1941 of the population then living seems to preclude the possibility of any human epidemic and indicates that these immune children must have received their infections from mosquitoes which had bitten wild animals. If this is so, the transference of virus from the wild mammals to the natives must be of fairly frequent occurrence. *A. africanus* is known to enter and bite in native plantations though in small numbers (Haddow, 1945b). *A. simpsoni* is also a possible vector, acquiring its infection either from monkeys in the canopy at the forest edge where it is known to be active occasionally in the close proximity of banana plantations (Haddow, personal communication) or from monkeys raiding plantations for food (Smithburn & Haddow, 1946).

General Description.

A general description of Bwamba has been given by Haddow (1945a) ; only a few pertinent details will be enlarged upon here.

The natives of Bwamba (called Baamba) are an agricultural people carrying on cultivation mainly of coffee, cotton, " dry " rice, bananas, cassava and colocasia. The first three are commercial crops, the last three for local consumption. Cultivation is largely of a shifting character ; virgin forest is constantly being felled and burned to provide new ground and after a few years' cultivation an area is usually allowed to lie fallow for a period of years before being again taken into use. Under these conditions, a dense growth of elephant grass (*Pennisetum purpureum* Schumach.) or of rapidly growing trees arises.

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The native huts are almost universally circular, 5–7 m. in diameter, of grass thatch on a frame of poles, sometimes of beehive pattern, sometimes with separate roof and walls. They may be partitioned internally by screens of canes. Always of poor construction, they are without windows but their walls are perforated by many adventitious apertures. Internal cooking fires are nearly always present and the huts are very dirty and sooty. Besides their human population, usually very overcrowded, they shelter dogs, goats and chickens by night. Water jars are common in the houses, but water is not in general stored in them for long periods as no part of the area is far from perennial streams.

The natives occupy the huts from about 20 hours Local Mean Time* until about dawn. Sometimes a few frequent them during the daylight hours, but for the most part they sit outside near the huts or in the shade of a " banda", a thatched shelter with its sides left open. In the evening, the meal is generally taken about sunset and, up till 20 hours, the natives congregate in or near the banda and sit in the dark until they go to bed. Work in the plantations, mainly by the adult women and men, is during the daylight hours, chiefly in the morning and early evening. The huts, the open space and the banda near them, and the plantation are the localities in which by far the major portion of the existence of the natives is passed. They do not spend much time in the forest and then generally only during daylight.

Three localities were used, two of which were in the valley of the Ntotoro river in the middle reach of its course from Ruwenzori to the Semliki river ; these will be referred to as Ntotoro West and East. The third, to be called Tokwe, was on the edge of the Semliki Crown Forest and was one of the localities in which the transmission of yellow fever virus was demonstrated during the 1948 outbreak (Smithburn & others, 1949). All these localities are at altitudes of about 750 m. on the floor of the Western Rift Valley.

The adjoining forest is virgin at Ntotoro and is composed almost entirely of African ironwood (*Cynometra alexandri* C. H. Wright). There was a group of five huts at Ntotoro West, surrounded by rank growth, and plantations of cassava, maize and banana, 40–60 m. from the forest and about 150 m. from the river. The population of these huts was about 10 adults and 18 children besides goats, dogs and fowls. There were two huts at Ntotoro East, so close to the forest edge that their backs were covered with forest creepers. They were about 100 m. from the river and were adjoined by a previously-cleared space which had been allowed to revert to elephant grass and rank growth. They had been unoccupied for a few days before being used for the catch. At Tokwe there were two huts about 70 m. from the forest edge, housing three people and surrounded by plantations of coffee, cotton and maize.

Methods.

" Knockdown " catches with pyrethrum spray were used in the huts at Ntotoro West at first. One catch was made daily for five successive days in each of four huts at 09, 15, 18 and 21 hours, the order being changed from day to day. These catches were made by occluding with cotton fabric the more extensive apertures in the walls, spreading squares of the same material to cover all horizontal surfaces, spraying with pyrethrum in kerosene, leaving for 15 minutes and then picking up all the insects on the cotton fabric. These catches were timed to provide information on resting insects in the morning (09 hours), and to cover the times of maximum biting activity in forest of possibly important species—*Aëdes* (*Stegomyia*) *apicoargenteus* (Theo.) (15 hours), *A. africanus* (18 hours) and *Taeniorhynchus* (*Mansonioides*) *africanus* (Theo.) (21 hours)—as known from previous work in forest (Haddow, Gillett & Highton, 1947). These catches were disappointing in the numbers of biting insects produced and were abandoned in favour of 24-hour baited catches, made by methods exactly similar to those described by Haddow, Gillett and Highton (1947), which allowed simultaneous comparison of the biting fauna in the hut with those of the space outside, the nearby plantations, and ground and canopy levels in the adjoining forest. It should be noted that in this method the insects are caught in small tubes as they alight on the skin, and it is not certain that all species do so to bite unless they are actually observed probing or are not caught immediately on alighting and are on subsequent examination found to contain blood. However, this reservation applies only to the rarer species. At Ntotoro West, teams of five native children were used as bait and all the stations described above were employed with the exception of the forest platform ; all the stations were within a circle of 100 m. diameter. At Ntotoro East, all stations were used and were within 35 m. of each other, except for that in the plantation which was sited as near as possible but which was 95–130 m. from the others ; the bait here was of four native children at each station. At Tokwe, again, all five stations were used and were within 130 m. of each other ; the bait was of three native children at each station.

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*The 24-hour clock is used throughout.

Microclimate of Huts.

The microclimatic conditions in forest, plantation and in the open have been discussed in detail by Haddow (1945b). In the present work, thermograph and hygrograph tracings were taken in the hut at Ntotoro West when it was being used for the 24-hour catch from 18th to 23rd October, 1947. The instruments were exposed in a sheltered part of the hut at a height of 60 cm. from the ground, but were not enclosed in screens. They were checked by means of a whirling psychrometer. The traces are broadly comparable with those of Haddow (*loc. cit.*) for exterior environments (open air, plantation and forest), his work being carried out in June and September, also during the more rainy part of the year.

The thermograph trace in the hut is a smooth one, devoid of the short-term fluctuations which are characteristic of Haddow's open air trace, *i.e.*, in this respect it most resembles his forest one. Maxima fell between 14 and 16 hours and minima regularly at about 07 hours. The extreme temperatures observed were 22°C. and 31°C. ; the mean daily range was 7·4°C. The times of maximum and minimum temperatures both tended to be delayed as compared with Haddow's records ; his latest minimum was 06 hours in plantation and in forest, and his latest maximum at 15 hours, in plantation and in the open. The mean daily range of temperature approximated to Haddow's findings for plantation and forest and was much less than his values in the open. The relative humidity trace shows short-term fluctuations of small amplitude similar to his findings in forest and plantation, and its general form also is similar. There was a period of high humidity (over 80 per cent. R.H.) from 16–21 hours until 10 hours. The relative humidity began to fall daily at about 08 hours, which is close to Haddow's finding for forest and later than either in plantation or in the open. Relative humidity fell below 70 per cent. daily at about noon and remained so for periods up to seven hours, the mean for the five days being 4·3 hours. This latter value is intermediate between Haddow's values in plantation and in the open. In general, therefore, the hut is an environment sheltered from the more marked climatic variations of the open and comparable in conditions more to forest or plantation. In light intensity, the huts are generally below even the forest environment, as the sooty walls and roof are highly absorptive in spite of the fact that there are usually many apertures.

The Species Encountered.

The nomenclature used follows Evans (1938) and Edwards (1941). In cases in which the females are not susceptible of exact determination, they are relegated to groups. Full names are given here and abbreviated ones will be used subsequently.

Anopheles (*Anopheles*) *coustani* Lav. (*s. str.*)
,, ,, ,, ,, var. *ziemanni* Grünb.
,, ,, ,, ,, West African var.
,, ,, *paludis* Theo.
,, ,, *implexus* (Theo.)
,, (*Myzomyia*) *nili* Theo.
,, ,, *funestus* Giles
,, ,, *hargreavesi* Ev.
,, ,, *demeilloni* Ev.
,, ,, *gambiae* Giles
,, ,, *pharoensis* Theo.
,, ,, *squamosus* Theo.
Harpagomyia taeniarostris Theo.
Ficalbia (*Mimomyia*) *plumosa* (Theo.)
,, (*Etorleptiomyia*) *mediolineata* (Theo.)
Taeniorhynchus (*Coquillettidia*) *maculipennis* Theo.
,, ,, *fuscopennatus* Theo.
,, ,, *aurites* Theo.
,, (*Mansonioides*) *africanus* (Theo.)
,, ,, *uniformis* (Theo.)
Aëdes (*Mucidus*) *nigerrimus* (Theo.)
,, ,, *grahami* (Theo.)
,, (*Finlaya*) *longipalpis* (Grünb.)
,, ,, *ingrami* Edw.
,, (*Stegomyia*) *aegypti* (L.)
,, ,, *simpsoni* (Theo.)
,, ,, *apicoargenteus* (Theo.)
,, ,, *fraseri* (Edw.)
,, ,, *dendrophilus* Edw.
,, ,, *africanus* (Theo.)
,, ,, *vittatus* (Big.)
,, (*Aëdimorphus*) *stokesi* Ev.
,, ,, *mutilus* Edw.
,, ,, *domesticus* (Theo.) group
,, ,, *tarsalis* (Newst.) group.
,, ,, *abnormalis* (Theo.) group
,, ,, *cumminsi* (Theo.)
,, (*Banksinella*) *circumluteolus* (Theo.)
,, ,, *palpalis* (Newst.)
,, (*Dunnius*) *albomarginatus* (Newst.)
Eretmapodites chrysogaster Graham group
,, *inornatus* Newst. group
Culex (*Culex*) *moucheti* Ev.
Haematopota vittata (Loew)
,, *furva* Aust.
Chrysops centurionis Aust.
Stomoxys sp. indet.
Glossina fusca congolensis Newst. & Ev.

Results.

The spray catches.

Forty of these catches were made, all at Ntotoro West, as follows: 8th–12th October, 1947, 20; 8th–9th July, 1948, 5; 3rd–7th August, 1948, 15. The biting insects yielded are set out below :—

REDUVIIDAE		2
PHLEBOTOMINAE		1
A. (*M.*) *funestus*	♂	89
	♀	526
A. (*M.*) *gambiae*	♂	95
	♀	636
H. taeniarostris	♀	1
T. (*M.*) *africanus*	♀	1
Culex sp. indet.	♀	1
Stomoxys sp. indet.		2
		1,354

A. (*M.*) *funestus* and *A.* (*M.*) *gambiae* are well known to use huts as daytime resting places, and this habit accounts for the marked numerical preponderance of these species. Although timed to coincide with the periods of maximum biting activity of suspect species, the catches yielded only a single specimen of *T.* (*M.*) *africanus*. It is therefore evident that none of them habitually use the huts as a resting place even if they enter to bite the occupants. *H. taeniarostris* is a non-haematophagous species. Neither Reduviids nor *Stomoxys* have been reported as biting in the forest despite extensive investigations (Haddow, Gillett & Highton, 1947) so that they are unlikely to be of any importance. The PHLEBOTOMINAE are insects of very short flight range and a single species is unlikely to be common to environments as separated as the forest and the hut.

The baited catches.

In two of the localities (Ntotoro West and Tokwe) the catches lasted for a few hours over five days, in the other (Ntotoro East) for five days exactly. The results of the initial few hours have been excluded in the former two cases for purposes of comparison ; there was no outstanding occurrence within these hours except that a single *A.* (*S.*) *aegypti* was taken at Ntotoro West, the only specimen taken throughout the work. The results of the three catches are shown in Tables I–III and the catch

TABLE I.

The results of a 24-hour catch lasting five days (18th–23rd October, 1947) at Ntotoro West, Bwamba, Uganda. Catches of each species in each of the environments frequented by man are divided into two components, that occurring during the daily period when the environment is or may be occupied by man (A), being separated from that when it is not so occupied (B). For definition of these periods see text.

Species or group	Catching station								Totals
	Forest floor		Plantation		Open space		Hut		
	A	B	A	B	A	B	A	B	
Anopheles coustani var. *ziemanni*	—	—	1	3	—	—	—	—	4
Anopheles coustani W. African var.	—	—	—	3	—	—	—	—	3
Anopheles paludis	3	2	1	10	—	—	—	—	16
Anopheles implexus	—	4	—	—	—	—	—	—	4
Anopheles nili	—	—	6	—	—	—	1	—	7
Anopheles funestus	9	2	49	36	13	16	252	186	563
Anopheles hargreavesi ...	—	—	—	1	—	—	—	—	1
Anopheles gambiae	3	5	16	35	10	25	101	21	216
Anopheles pharoensis... ...	—	—	1	2	—	—	—	—	3
Taeniorhynchus fuscopennatus	9	43	7	27	—	—	—	—	86
Taeniorhynchus africanus ...	3	16	56	134	2	9	4	—	224
Taeniorhynchus uniformis ...	1	4	66	174	4	12	1	1	263
Aëdes simpsoni	1	2	52	—	3	—	—	—	58
Aëdes dendrophilus	1	—	—	—	—	—	—	—	1
Aëdes africanus	1	1	—	—	—	—	—	—	2
Aëdes vittatus	—	—	—	—	1	—	—	—	1
Aëdes stokesi	—	1	—	—	—	—	—	—	1
Aëdes mutilus...	—	1	—	—	—	—	—	—	1
Aëdes domesticus group ...	—	1	—	—	—	—	—	—	1
Aëdes tarsalis - abnormalis group	47	21	—	1	5	—	—	—	74
Aëdes cumminsi	13	14	1	6	—	—	—	—	34
Aëdes circumluteolus	229	74	10	13	63	2	—	1	392
Aëdes palpalis	161	60	—	—	5	—	—	—	226
Eretmapodites chrysogaster group	17	6	—	—	1	—	—	—	24
Eretmapodites inornatus group	1	—	1	—	—	—	—	—	2
*Culex moucheti**	17	7	—	—	5	—	—	—	29
Culex spp. indet.	11	12	1	15	1	4	1	—	45
Haematopota vittata	1	—	—	—	—	—	—	—	1
Haematopota furva	1	—	—	—	—	—	—	—	1
Glossina fusca congolensis ...	1	—	—	—	—	—	—	—	1
Totals	530	276	268	460	113	68	360	209	2,284
Number of species or groups	20	19	14	14	12	6	6	4	30

*Separated from *Culex* spp. indet. for last four days of catch only.

TABLE II.

The results of a 24-hour catch lasting for five days (17th–22nd December, 1947) at Ntotoro East, Bwamba, Uganda. For explanation see legend of Table I and text.

Species or group	Catching station									Totals
	Forest canopy	Forest floor		Plantation		Open space		Hut		
		A	B	A	B	A	B	A	B	
Anopheles coustani (s. str.) ...	—	—	—	—	—	—	1	—	—	1
Anopheles coustani var. *ziemanni* ...	—	—	—	1	1	—	—	—	—	2
Anopheles paludis ...	—	—	—	3	—	1	—	—	—	4
Anopheles implexus ...	—	4	8	—	—	2	—	—	—	14
Anopheles funestus ...	—	—	1	2	5	—	—	—	2	10
Anopheles hargreavesi	—	—	2	—	3	2	1	—	—	8
Anopheles demeilloni ...	—	—	—	1	—	—	—	—	—	1
Anopheles gambiae ...	—	16	33	40	45	28	34	24	11	231
Taeniorhynchus maculipennis ...	—	1	—	—	—	—	—	—	—	1
Taeniorhynchus fuscopennatus ...	11	10	30	1	12	12	22	—	—	98
Taeniorhynchus aurites	1	1	—	—	—	—	—	—	—	2
Taeniorhynchus africanus ...	10	6	20	50	86	7	18	2	2	201
Taeniorhynchus uniformis ...	4	—	—	14	17	3	2	—	—	40
Aëdes nigerrimus ...	2	—	—	—	—	—	—	—	—	2
Aëdes grahami ...	2	—	—	—	—	—	—	—	—	2
Aëdes longipalpis ...	8	1	—	—	—	—	—	—	—	9
Aëdes simpsoni ...	8	10	—	238	—	12	—	—	—	268
Aëdes apicoargenteus ...	3	—	—	—	—	—	—	—	—	3
Aëdes fraseri ...	—	1	—	—	—	—	—	—	—	1
Aëdes africanus ...	7	1	—	—	—	—	—	—	—	8
Aëdes stokesi ...	—	1	—	—	—	—	—	—	—	1
Aëdes domesticus group	—	1	—	—	—	—	—	—	—	1
Aëdes tarsalis-abnormalis group ...	—	41	4	—	—	6	—	—	—	51
Aëdes cumminsi ...	—	36	12	—	1	15	40	—	—	104
Aëdes circumluteolus ...	—	26	1	4	2	3	2	—	—	38
Aëdes palpalis ...	—	15	—	—	—	—	—	—	—	15
Eretmapodites chrysogaster group ...	—	3	—	—	—	—	—	—	—	3
Culex spp. indet. ...	5	41	3	5	7	4	4	1	—	70
Chrysops centurionis ...	5	—	—	—	—	—	—	—	—	5
Totals	66	215	114	359	179	95	124	27	15	1,194
Number of species or groups	12	18	10	11	10	12	9	3	3	29

of each species in each of the environments frequented by man is there divided into two components : one, the numbers occurring during the daily period in which the environment is or may be occupied by natives, and the other, the numbers occurring during unoccupied periods. These periods were considered to be :—

Forest floor and plantation : occupied, 05 to 19 hours; unoccupied, 19 to 05 hours.

Open space : occupied, 05 to 20 hours; unoccupied, 20 to 05 hours.

Hut : occupied, 20 to 06 hours; unoccupied, 06 to 20 hours.

In considering these periods, preference is given to the occupied ones so that all the times at which the environments may be occupied are included. For example, work in the plantations may go on until it is nearly dark so that the natives there may be exposed to the bites of insects active in the twilight ; the occupied period is therefore extended to one hour after sunset.

Transference of yellow fever virus from a forest mammalian host to a native by a biting insect may theoretically occur in several ways. The infecting mammal is almost certain to be a monkey (Haddow & others, 1947), which may be bitten in the forest canopy or while visiting the forest floor or the plantation. The insect thus infected may then subsequently bite man in one of the environments which he frequents. As far as the acquisition of infection by the insect from monkeys in the forest canopy is concerned, it is necessary to consider the catch for the whole 24-hour period, as the important monkey species are mainly or entirely arboreal and may be found in the canopy at any time. In considering the acquisition of infection from monkeys on the forest floor or in plantation, only the daylight

TABLE III.

The results of a 24-hour catch lasting for five days (9th–14th July, 1948) at Tokwe, Bwamba, Uganda. For explanation see legend of Table I and text.

Species or group	Catching station									Totals
	Forest canopy	Forest floor		Plantation		Open space		Hut		
		A	B	A	B	A	B	A	B	
Anopheles paludis ...	—	—	1	2	3	2	—	—	—	8
Anopheles implexus ...	—	1	—	—	—	—	—	—	—	1
Anopheles funestus ...	—	—	—	—	—	—	—	4	—	4
Anopheles gambiae ...	—	—	—	—	2	1	10	5	—	18
Anopheles pharoensis...	—	—	—	1	7	2	4	—	—	14
Anopheles squamosus...	—	—	—	—	1	1	—	—	—	2
Ficalbia plumosa ...	—	—	—	—	—	—	1	1	—	2
Ficalbia mediolineata	—	1	—	1	2	—	8	12	—	24
Taeniorhynchus fuscopennatus	1	—	—	—	—	—	—	—	—	1
Taeniorhynchus aurites	10	—	—	—	—	—	—	—	—	10
Taeniorhynchus africanus	17	11	21	46	369	56	200	46	2	768
Taeniorhynchus uniformis	5	3	2	10	34	14	36	1	2	107
Aëdes nigerrimus ...	1	—	—	—	—	—	—	—	—	1
Aëdes grahami ...	1	—	—	—	—	—	—	—	—	1
Aëdes longipalpis ...	13	—	—	—	—	—	—	—	—	13
Aëdes ingrami ...	—	1	—	—	—	—	—	—	—	1
Aëdes simpsoni ...	1	—	—	7	—	1	—	—	—	9
Aëdes apicoargenteus	1	—	—	—	—	—	—	—	—	1
Aëdes africanus ...	7	1	—	—	—	—	—	—	—	8
Aëdes stokesi	—	1	—	—	—	—	—	—	—	1
Aëdes tarsalis group ...	—	2	—	1	—	—	—	—	—	3
Aëdes cumminsi ...	—	6	5	1	5	1	2	1	—	21
Aëdes circumluteolus ...	—	5	1	13	13	6	3	—	1	42
Aëdes albomarginatus	—	1	—	—	—	—	—	—	—	1
Eretmapodites chrysogaster group ...	—	4	—	—	1	—	—	—	—	5
Culex spp. indet. ...	14	39	15	10	16	9	10	5	—	118
Glossina fusca congolensis	—	—	1	—	—	—	—	—	—	1
Totals	71	76	46	92	453	93	274	75	5	1,185
Number of species or groups	11	13	7	10	11	10	9	8	3	27

component is admitted, as visits made by monkeys to these environments are confined to the daylight hours. By night, all species sleep in the trees. Comparisons of the insects biting in the forest canopy throughout the 24 hours, and on the forest floor and in plantation by daylight, with those biting on the forest floor, in plantation, in the open space and in the hut during their usual periods of occupation by natives, are therefore likely to indicate the vector or vectors most probably concerned.

Theoretical considerations in the assessment of the results are discussed below. It may be postulated that of two species of biting insect, X and Y, in a circumscribed area in which a catch is made, the total populations are p and p′, respectively. Let the total numbers ready to bite on any day in these populations be respectively represented by n and n′ for the two species. As the bait is present throughout the 24-hour period and the area concerned is a small one, it may be assumed that those ready to bite do so and the total numbers taken during that period approximate to n and n′. These are, however, only a fraction of the total population, the remainder, for mosquitoes at least, being made up of those individuals which are developing eggs after a previous blood meal, ovipositing or engaged in other activities. Of the total daily numbers biting (n and n′), let a and b respectively represent the daily totals of species X taken biting in two different environments during the periods of occupation and a′ and b′ fulfil the same purpose for species Y. The proportion of the total population of species X biting in the first environment is therefore a/p for species X and a′/p′ for species Y. Similarly, the corresponding proportions for the second environment are b/p and b′/p′ respectively. Neglecting considerations other than those of chance, the proportions of the total populations biting in the first environment and subsequently in the second are therefore represented by ab/p^2 and $a'b'/p'^2$ for species X and Y, respectively. The actual numbers will be these terms multiplied by the total populations, *i.e.*, ab/p and a′b′/p′, respectively. If z

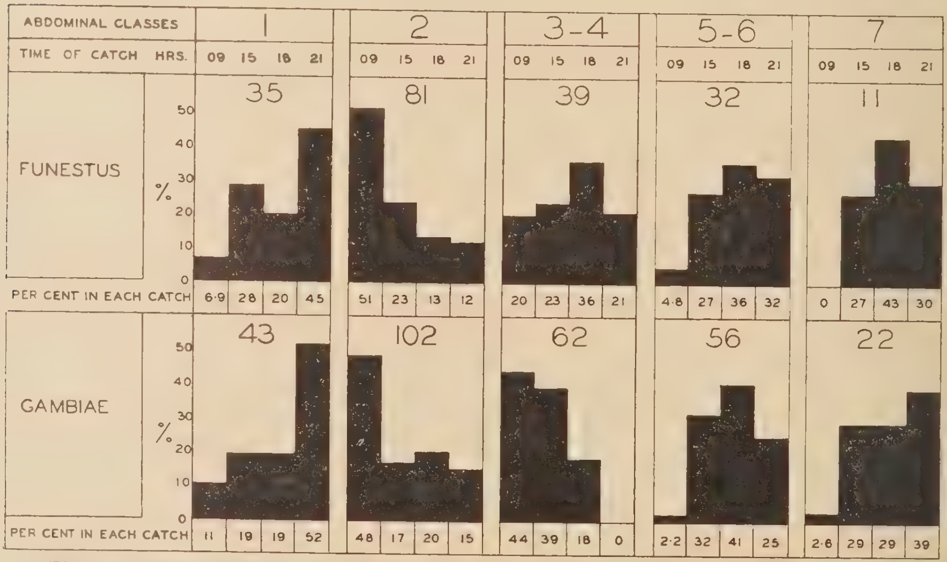

Fig. 1.—The distribution of the abdominal classes of Grassi and Sella in female *A. gambiae* and *A. funestus* taken in " knockdown " catches in native huts at Ntotoro West, Bwamba, Uganda ; 9th–12th October, 1947. After standardisation to equal numbers of mosquitoes at each catch time, the distributions of the abdominal classes through the four catches are expressed as percentages. Classes 3 and 4, and 4 and 5, are grouped together. The large numerals in each compartment show the numbers on which each group of histograms is based.

and z′ represent respectively the proportions of species X and species Y which are ready to bite on any given day, then z will equal n/p and z′ will equal n′/p′. Substitution of the values of p and p′ derived from these in the foregoing gives the actual numbers biting in both environments as abz/n and a′b′z′/n′. All these terms may be estimated from the results of the catches with the exceptions of z and z′, the proportions which the biting populations bear to the total populations. While this term may be assumed to be approximately constant for any single species and therefore neglected in comparing the same species through several environments, it is less defensible to make such an assumption for the comparison of different species. However, most of the species to be compared are haematophagous mosquitoes which form a fairly homogeneous group as far as their bionomics are concerned. The value of z will depend largely on their reproductive cycle. In general they may be expected to take a blood-meal, develop eggs, oviposit, and then shortly afterwards be ready to bite again. The proportion ready to bite will therefore bear a close relation to the duration of this cycle. Some indication of its duration in *A. gambiae* and *A. funestus* may be obtained from the "knockdown" catches. For the last four days (16 catches) of the series at Ntotoro West, the abdominal classes of Grassi and Sella (Gater, 1935) of the females obtained were recorded. If the catches made at the four different times are standardised to equal numbers and the distribution of each class through the four catches is then examined, the arrangement shown in fig. 1 results. The incidence of the various abdominal classes is similar in the two species but the trend is clearer in the case of *A. gambiae*, which is discussed. Unfed females (Class 1) occur in all the catches but mainly in that at 21 hours. Females in Class 2 (newly-fed) are mainly to be found in the first catch (09 hours). The majority of those in Classes 3 and 4, and 5 and 6, occur in the catches at 09 and 15 hours, and 15 and 18 hours, respectively. Females with mature ovaries (Class 7) happen mainly in the later catches, reaching a maximum in that at 21 hours. It is evident that digestion of blood and development of ovaries takes place concurrently through the day in a large section of the population in the huts, implying that the mosquitoes with fully developed ovaries, found in the hut in the evening, fed the previous night. Oviposition seems to take place after 21 hours but before 09 hours the following day, as by the later time the proportion of females with fully-developed ovaries has fallen to a very low level. It is probable that these females after oviposition are again ready to feed the following night and compose part of the high proportion of unfed females occurring in the catch at 21 hours. The complete cycle of this species therefore appears to occupy 48 hours, each female feeding every second night ; in this case z would approximate to 0·5. It has been shown above that the climatic conditions in the hut approximate to those in forest or plantation, so that mosquitoes in these environments may be expected to have cycles not greatly differing in duration from that of *A. gambiae*. In any case, these cycles are likely to be short so that z will therefore generally be a large fraction of the total population. It seems legitimate to neglect this term in comparing the potentialities of different mosquito species, at least if the differences found in the expression ab/n are large. Table IV shows the maximum values of ab/n, calculated to two significant figures, obtained in various environmental combinations from all three catches. The maximum values have been selected as indicative of the maximum potentiality of species and routes of transference, as there were marked variations in abundance of species from catch to catch. Values are also given for forest floor with forest floor and for plantation with plantation, postulating that the transference may take place within these environments from monkeys to visiting or working natives. They are derived in a similar manner, using the squares of the biting totals in occupied periods (a^2/n). It should be borne in mind that the values given are developed in a manner analogous to, rather than identical with, considerations of pure probability, and are based on the preliminary assumptions outlined above. They should be regarded only as indices to actuality and should be interpreted in the light of whatever other information is available.

TABLE IV.

Comparisons of the common contacts of biting insects on hosts (native children) in pairs of environments. The figures given are indices representing the numbers of insects biting first in one environment and a second time in the other. For method of calculation see text.

Species or group	Forest canopy and				Forest floor and				Plantation and		
	forest floor	plantation	open space	hut	forest floor	plantation	open space	hut	plantation	open space	hut
Anopheles coustani var. *ziemanni* ...	—	—	—	—	—	—	—	—	0·10	—	—
Anopheles paludis ...	—	—	—	—	0·11	0·038	—	—	0·45	0·15	—
Anopheles implexus	—	—	—	—	0·23	—	0·11	—	—	—	—
Anopheles nili ...	—	—	—	—	—	—	—	—	1·0	—	0·17
Anopheles funestus...	—	—	—	—	0·029	0·16	0·042	0·81	0·85	0·23	4·4
Anopheles demeilloni	—	—	—	—	—	—	—	—	0·20	—	—
Anopheles gambiae...	—	—	—	—	0·22	0·55	0·39	0·33	1·4	0·97	1·5
Anopheles pharoensis	—	—	—	—	—	—	—	—	0·066	0·029	—
Ficalbia mediolineata	—	—	—	—	0·0083	0·0083	—	0·10	0·0083	—	0·10
Taeniorhynchus maculipennis ...	—	—	—	—	0·20	—	—	—	—	—	—
Taeniorhynchus fuscopennatus ...	0·22	0·022	0·27	—	0·20	0·15	0·24	—	0·11	0·024	—
Taeniorhynchus aurites	0·10	—	—	—	0·10	—	—	—	—	—	—
Taeniorhynchus africanus	0·60	0·50	0·25	0·20	0·036	0·30	0·16	0·13	2·8	0·67	0·55
Taeniorhynchus uniformis	0·28	0·28	0·13	0·0093	0·017	0·056	0·079	0·0056	3·3	0·26	0·050
Aëdes longipalpis ...	0·040	—	—	—	0·022	—	—	—	—	—	—
Aëdes ingrami ...	—	—	—	—	0·20	—	—	—	—	—	—
Aëdes simpsoni ...	0·060	1·4	0·072	—	0·075	1·8	0·090	—	42	2·1	—
Aëdes fraseri ...	—	—	—	—	0·20	—	—	—	—	—	—
Aëdes dendrophilus	—	—	—	—	0·20	—	—	—	—	—	—
Aëdes africanus ...	0·18	—	—	—	0·10	—	—	—	—	—	—
Aëdes stokesi ...	—	—	—	—	0·20	—	—	—	—	—	—
Aëdes domesticus group	—	—	—	—	0·20	—	—	—	—	—	—
Aëdes tarsalis-abnormalis group ...	—	—	—	—	6·6	0·13	0·96	—	0·066	—	—
Aëdes cumminsi ...	—	—	—	—	2·5	0·076	1·04	0·057	0·0095	0·0095	0·0095
Aëdes circumluteolus	—	—	—	—	27	1·2	7·4	—	0·80	0·37	—
Aëdes palpalis ...	—	—	—	—	23	—	0·71	—	—	—	—
Aëdes albomarginatus	—	—	—	—	0·20	—	—	—	—	—	—
Eretmapodites chrysogaster group ...	—	—	—	—	2·4	—	0·14	—	—	—	—
Eretmapodites inornatus group ...	—	—	—	—	0·10	0·10	—	—	0·10	—	—
Culex moucheti ...	—	—	—	—	2·5	—	0·73	—	—	—	—
Culex spp. indet. ...	0·93	0·24	0·21	0·12	4·8	0·66	0·59	0·33	0·17	0·15	0·085
Haematopota vittata	—	—	—	—	0·20	—	—	—	—	—	—
Haematopota furva...	—	—	—	—	0·20	—	—	—	—	—	—
Glossina fusca congolensis	—	—	—	—	0·20	—	—	—	—	—	—

Discussion.

Considering the high level of immunity present in the monkey population of Bwamba (Haddow, Smithburn & others, 1947), the short period during which a susceptible animal is capable of infecting the insect host and the apparent frequency with which transference has taken place, it seems almost certain that the insect vector connecting the forest and the human cycles should be found among the less rare insects common to the environments occupied or visited by monkeys and man, and therefore among those which yield high values in Table IV.

The highest value (42) is given by *A. simpsoni* in plantation. This species is a known vector of yellow fever which has been found infected in the field in Bwamba (Mahaffy & others, 1942 ; Smithburn & Haddow, 1946). The environment indicated is also a very likely one ; plantation is frequently raided by monkey, in Bwamba particularly by redtail (*Cercopithecus ascanius schmidti* Matschie) and *A. simpsoni* is known to bite in the crowns of the banana trees at the level of the fruit clusters as well as at ground level (Haddow, 1945c). The adult natives spend long periods each day in the plantations, which are also much frequented by the children. Only moderate values are found for *A. simpsoni* in other environmental combinations. The highest of these (2·1) is between plantation and open space, indicating a route of infection, though a much less effective one, for individuals not visiting the plantation. The figure between forest canopy and plantation, another suspected route, is 1·4, only about a thirtieth of the value for plantation. Between the forest floor, an environment much less frequented both by monkey and man, and plantation it is not much larger (1·8). There seems little doubt that the main route of transference is by *A. simpsoni* from raiding monkey in the plantation to the human population working in the same environment.

The second highest index (27) is that yielded by *A. circumluteolus* on the forest floor. However, the forest floor is an environment to which only brief visits are made by the natives and does not compare in duration of occupation with the plantation. On this account alone the index of 27 is probably much less important than at first appears. As far as is known, the capacity of this species to maintain yellow fever virus in the laboratory has not yet been investigated. It has not been suspected as a vector in the field on account of its scarcity in forest at any but ground level (Haddow, Gillett & Highton, 1947). The capability of the species to transmit in the laboratory is perhaps worthy of investigation. It yields also a fairly high index (7·4) between the forest floor and the open space. Even if it should be found incapable of acting as a vector for yellow fever, it should be borne in mind as possibly of importance in relation to other forest viruses. The third highest index (23) is shown by the closely-allied species *A. palpalis* to which similar remarks apply.

The *A. tarsalis-abnormalis* group yields the fourth highest index (6·6), but only in the unfavourable environment of the forest floor. These mosquitoes also have not incurred suspicion as vectors in the field owing to their scarcity in biting catches at other than ground level (Haddow, Gillett & Highton, 1947).

Indices below this level are given by a considerable number of species, most of which only produce very low values. It seems unnecessary to discuss them in detail except for those which, for one reason or another, have already incurred suspicion as yellow fever vectors. *T. africanus* has been shown to act as a vector in laboratory studies (Philip, 1930), though the other species of the subgenus, *T. uniformis*, does not appear to do so (Kerr, 1932). In the present work, *T. africanus*, though widespread through all the environments, yielded only inconsiderable indices, except in the plantation (2·8), between the plantation and the open space (0·67) and the hut (0·55) and between the forest canopy and the plantation (0·50). The closely-allied species, *T. uniformis*, shows only small indices except in the plantation (3·3). These species cannot be entirely dismissed from consideration, but comparison of their indices with that for *A. simpsoni* in the plantation leads to the conclusion that they must be of minor importance. Further, during the period and in the area of active yellow fever transmission in the forest in Bwamba in 1948, which has been described by Smithburn & others (1949), yellow fever virus was not demonstrated in these two species although 2,428 were inoculated into susceptible rhesus monkeys (*Macaca mulatta* Zimmermann) ; at the same time virus was repeatedly shown by the same methods in a total of 2,040 *A. africanus*.

A. africanus is known to be of great importance in the transmission of yellow fever virus among monkeys in Bwamba forests (Smithburn & others, 1949). The species yields, in the present work, small indices between the forest canopy and the forest floor (0·18) and on the forest floor (0·10). *A. africanus* was not taken in the plantation but the work of Haddow (1945b) has shown that the species may on occasion bite man there. It is worth while to attempt a comparison with his figures by the method of indices. In 15 24-hour catches at two stations 40 yards apart, he took 15 *A. africanus* at the station on the forest floor and 57 at the one in the plantation, mean daily catches of 1·0 and 3·8, respectively. No catch was made in the forest canopy, so that it is necessary to estimate what it might have been. Haddow, Gillett and Highton (1947) give the results of simultaneous catches on the forest floor and in the forest understorey and canopy in two localities, in both dry and rainy seasons. Their figures show that *A. africanus* may be as much as 24 times as numerous at two stations in the canopy as at a single one at ground level. Taking this, their highest value, as a working basis, the canopy in the other work, had it been included, might have produced a mean daily catch of 24 *A. africanus*. Assuming also that *A. africanus* would not have occurred in catches in a hut or an open space, where it has not yet been recorded as biting by either Haddow and his co-workers or the writer, the indices for the species would have been :

> Between the forest canopy and floor, 0·83 ; between the forest canopy and the plantation, 3·2 ; on the forest floor, 0·35 ; between the forest floor and the plantation, 0·13 ; in the plantation, 0·50 ; all other indices, zero.

The index between forest canopy and plantation especially merits serious consideration, pointing to another possible route of infection of the human population. However, in comparison with the very high index for *A. simpsoni* in plantation, it is probably of minor importance.

A. stokesi has been found to transmit in the laboratory (Bauer, 1928), but it yields only a very small index (0·20) in the unfavourable environment of the forest floor. *E. chrysogaster* has also (Bauer, 1928) been incriminated as a laboratory vector. The group including this species yields a moderate index (2·4) on the forest floor and a small one (0·14) between the forest floor and the open space. It also is probably of only small importance.

Some species of the genus *Culex* have been found capable of transmitting yellow fever virus in the laboratory by Kerr (1932) and Davis (1933) but no high index was found except on the forest floor (4·8). Further, as the indices are calculated for a group of species, much lower values would probably apply to the several constituent species could they be separated and their indices calculated individually. This remark, of course, applies also to the other group indices.

Eleven species do not enter Table IV and are therefore even less worthy of consideration, but it should be noted that two of these were of the subgenus *Stegomyia* of *Aëdes*. The first, *A. apicoargenteus*, failed to transmit in laboratory experiments (Bauer, 1928) ; the other, *A. vittatus*, was found by Philip (1929) to act as a vector under these conditions. It is, however, an extremely rare species in Bwamba, the single specimen taken in the present work being the first, and as yet only, record. The rarity of *A. aegypti* as a mosquito attacking man in Bwamba, already reported by Haddow (1945a), is confirmed ; it also is on this account considered to be of no practical importance.

It should be pointed out that the present work employed stationary teams of native children as baits to sample the biting insect fauna in the various environments. A somewhat different situation might have appeared if moving baits, imitating more closely the habits of the natives in forest, had been used. Nevertheless, the forest floor is considered to be the least important environment as far as the native population is concerned, as individuals spend but little time there in comparison to that spent in the other environments.

In conclusion, it seems that by far the most important route of transference of yellow fever virus is from an infective monkey, probably a redtail (*C. a. schmidti*), raiding a plantation for food and there being bitten by *A. simpsoni* which subsequently passes the virus to man. Monkeys immune to yellow fever are widespread in Uganda, but immunity in the native population is more sporadic ; a highly immune monkey population may coexist in an area with natives among whom few or no immunes are to be found. The present work suggests that the proportion of immunes among the native population is to be closely related to the proximity of plantation to forest and to the local prevalence of *A. simpsoni*. Therefore, as this species breeds almost exclusively in the leaf axils of certain plants cultivated for food, it is related also to the local crops. A second explanation is the local prevalence of a monkey species which lives mainly in the forest canopy but which also frequently raids plantations for food. This explanation, however, is less likely to be true, at least in Uganda, since the redtail monkey, the worst offender in this respect in Bwamba, is widespread throughout the territory. The possibility of largely preventing the infection of the native population with yellow fever in such a locality as Bwamba is indicated—most simply by separating plantation from forest and by discouraging such crops as " gonja " banana and colocasia whose leaf axils have been shown by several workers to provide the main breeding places of *A. simpsoni* (Haddow, 1948).

Summary.

The habits and habitations of the natives in the Bwamba lowlands, Uganda, are briefly described.

A series of catches designed to assess the relative importance of the various species of biting insect and of various routes of transference in the transmission of yellow fever virus from the monkey population of the forest to the nearby human population is reported.

The microclimate in a native hut is discussed.

The species of biting insect encountered are listed, the results of the catches given, and the relative importance of the different species and routes assessed by means of indices.

It is concluded that yellow fever virus is most likely to be transferred to the native population by *A.* (*S.*) *simpsoni* in plantations, the infection in this insect being derived from forest monkeys which raid the plantations for food.

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THE SELECTION AND REARING OF LEAF-EATING INSECTS FOR USE AS TEST SUBJECTS IN THE STUDY OF INSECTICIDES.

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(Plate XII.)

Abundant evidence is available to show that, to a varying extent, all insecticides are specific in their action on insect species. A high degree of specificity may be a desirable property since an insecticide which is toxic to a certain test species whilst leaving other insects, particularly beneficial insects, unaffected, has many advantages over one which is widely toxic to both harmful and beneficial species. Thus, in the study of insecticidal action and particularly in the search for new insecticides it is important that the specificity of the insecticide should be examined. For such work, it is necessary to rear a range of insect species in the laboratory using techniques which are capable of maintaining healthy individuals of uniform resistance.

This paper deals with the problem of the selection and rearing of certain mandibulate leaf-eating insects. Rearing methods for such insects have been described by workers in the United States, and Waters (1943) gives a valuable survey of techniques used for rearing various plant-feeding species found in that country. The importance of controlled environmental conditions is emphasized (Waters, 1937) though at present there is little data available regarding optimum conditions of temperature, humidity and lighting for development of plant-feeding insects. Illustrations of cages and a summary of rearing methods are given by Peterson (1947), whilst papers by Swingle (1935) and Swingle and others (1941) deal more fully with rearing methods for certain leaf-eating insects found in the United States.

The aim of the present work has been to find several leaf-eating species from the Orthoptera, Lepidoptera, Hymenoptera and Coleoptera which as far as possible satisfy certain requirements—notably: (1) The food-plant must be available in large quantities throughout the year. This greatly limits the choice of test insects because relatively few plant species can be grown successfully in winter. (2) The insect must be easy to rear; adults should breed well, larvae should not be cannibalistic or susceptible to disease particularly under crowded conditions, and continuous breeding should not lower the resistance of the stock. (3) The insect should be available throughout the year. Most plant-feeding species spend the winter in diapause and some remain in this condition for at least six months in the year; their value as test subjects is limited unless diapause can be prevented or broken.

Some of the environmental factors affecting insect development have been studied in an attempt to determine the optimum conditions for rearing. Also simple rearing techniques have been developed which as far as possible can be standardised for a number of insect species and which require the minimum amount of handling of the insects.

It is proposed to discuss some of the factors concerned in the rearing of plant-feeding insects and then to give a general description of rearing procedures, followed by a more detailed examination of the various insect species that have been studied.

As regards the factors affecting the rearing of plant-feeding insects, it was not possible to examine a number of insect species individually. Therefore two representative species were chosen for special study—the Tomato Moth, *Diataraxia oleracea* (L.)

and the Mustard Beetle, *Phaedon cochleariae* (F.). Some studies were also made on the Diamond Back Moth, *Plutella maculipennis* (Curt.).

The Food-plant.

Relatively few species of food-plant can be raised in quantity throughout the year and even the most satisfactory, such as brassica species, show much variation in texture and physiological condition according to the time of the year, a factor which may cause variation in vigour of insects feeding on them. Although no difference in resistance to insecticides which can be attributed to this factor has been observed with the leaf-eating insects so far studied, it is clear that some attempt should be made to standardise the condition of the food-plant, or at least to make it more uniform.

The main difficulty encountered is to induce normal plant growth during winter, since under winter conditions of illumination most plants become etiolated unless raised at temperatures less than about 12°C. when the growth rate is generally too slow to be practicable. It is well known that if winter daylight is supplemented by artificial light, plant growth is much improved at higher temperatures (Brandon, 1946). Initial growth of large-seeded species and of tubers and root stocks may be obtained by using only artificial illumination from 100-w. Tungsten lamps (Waters, 1937). However, rapid deterioration occurs after formation of the first two to three leaves and it is felt that artificial illumination as a substitute for daylight cannot be considered satisfactory unless it will maintain normal vegetative growth of the plant.

Preliminary experiments have shown that sturdy and well coloured turnip plants can be grown from seed in a constant temperature room at 18°C. using artificial illumination from a pair of 400-w. Neon floodlighting lamps for 16 hours per 24 hours. Under similar conditions of temperature, healthy plants of tomato and turnip were also grown from seed under a 450-w. Mercury lamp. Under both types of lamps, cabbage plants produced abnormally small leaves and tended to become etiolated. It would seem therefore, that some plant species can be raised satisfactorily under controlled conditions of temperature and illumination, but, in the absence of knowledge on problems of illumination and plant growth it is felt that the greatest value of artificial light in rearing plant-feeding insects is (1) to supplement daylight in the glasshouse and thus induce satisfactory growth of the food-plant during winter, and (2) in insect rearing rooms to maintain the health of the food plant whilst it is being fed to the insects. A battery of 2–4 80-w. daylight fluorescent tubes suspended above the plants has proved satisfactory for these purposes.

Apart from availability, the choice of food-plants depends on a number of factors. Many insects are capable of feeding on a range of plant species, but clearly the most suitable food-plants are those which induce the lowest mortalities and the most vigorous and uniform development. The Mustard Beetle, *Phaedon cochleariae,* may be reared on mustard, turnip, cabbage or kale of which the two latter are probably more readily available in quantity throughout the year. To determine the most suitable food-plant, batches of 50 larvae from a stock reared for the previous two years on turnip were reared on the above plants in a constant temperature room at 24°C. The mortalities during the larval and pupal stages and the average weights of the resulting adults (Table I) suggest that mustard is the most suitable.

TABLE I.

Food-plant	Per cent. mortality to adult stage	Average wt. of newly emerged adults (mg.)
Mustard	18	4·97
Turnip	32	4·31
Cabbage	50	4·27
Kale	64	4·06

The effect of larval food-plant on the fecundity of the resulting adults was not determined though it is of interest that Kozhanchikov and others (1936) demonstrated considerable differences in fecundity of the adults of *Agrotis segetum* (Schiff.) depending on larval food-plant.

To determine the influence of food-plant during the adult stage, batches of newly emerged *P. cochleariae* adults, from larvae reared on turnip, were provided with turnip, brussels sprouts and kale for food and oviposition. Table II shows that the food-plant greatly influences fecundity. The predisposition of the adults towards turnip, the food-plant used for rearing previous generations, may have been the cause of the differences, though possibly the difficulty experienced by the adult beetles in gripping the smooth surface of cabbage and kale foliage was in part the reason for the unsuitability of these two plants. Mustard was not studied because, at the time it was not realised that this food-plant was notably different from turnip.

TABLE II.

The effect of certain food-plants on adults of *P. cochleariae*.

Food-plant	No. of insects	Total leaf area consumed (sq. cm.)	Total no. of eggs laid
Turnip	20	222	2,992
Brussels sprouts	20	143	832
Kale	20	100	754

Preliminary experiments with larvae of *Diataraxia oleracea* confirmed the observation of Speyer and Parr (1948) that larvae can be reared solely on foliage of tomato-variety Potentate. However, like other "noctuids", *D. oleracea* larvae develop satisfactorily on a wide range of food-plants including brassicas. Brassica species, unlike tomato, are of a suitable shape and size for rearing cages and moreover are readily available throughout the year. With cabbage as food-plant, there are usually five or six larval instars. On tomato (Speyer & Parr, 1948) there are six or seven and rarely five instars. Perhaps this indicates that tomato is less satisfactory as a food-plant. *D. oleracea* larvae appear to develop equally satisfactorily on all common brassicas, but preliminary experiments were carried out to determine whether such nearly related species of food-plant had a noticeable effect on vigour as shown by resistance of the larvae to an insecticide. Batches of *D. oleracea* larvae were reared from hatching on foliage of cabbage, brussels sprouts, curly kale and turnip in a constant temperature room at 24°C. No difference in rates of development was observed. Four to five days after ecdysis into the last instar (about 24 days after hatching) the larvae were sprayed with 0·2 per cent. w/v DDT in an emulsion containing 5 per cent. v/v benzene and 0·05 per cent. w/v cyclohexylamine dodecyl sulphate using the Potter tower (Potter, 1941). Average weights of the larvae with kills recorded by 0·2 per cent. DDT are shown in Table III.

TABLE III.

Effect of certain food-plants on the resistance of last instar *D. oleracea* larvae to DDT as a contact insecticide.

Larval food-plant	No. of insects	Average wt. 4–5 days after ecdysis (gm.)	Per cent. kill by 0·2 per cent. DDT emulsion
Cabbage (January King) ...	64	0·48	63
Brussels sprouts	73	0·51	58
Curly kale	64	0·51	59
Turnip	46	0·52	46

The data show small differences, which, if significant, could only be resolved by more detailed experiments. However, it has been shown with other insect species that certain food-plants have a marked effect on resistance to insecticides (Markos & Campbell, 1943). Therefore standardisation is desirable where possible.

The food may be presented to the insect either as cut foliage or as potted plants. The former method is more economical for large insects, while the latter is more suitable for smaller insect species (and early instars of large insects) because the use of potted plants largely eliminates the tedious and damaging process of handling small insects. For cut foliage (Pl. XII, fig. 2) long petiolated varieties are required and thus, for example, brussels sprouts is more suitable than cabbage. If potted plants are used, compact leaved varieties such as hearted cabbage plants hold the wet insect faeces in pockets of stagnant air between the leaves and as a result have caused disease among populations of *Plutella maculipennis* larvae reared under these conditions. If open headed varieties of food-plants are used (Pl. XII, fig. 1), the open growth allows fresh air to move freely between the leaves while faeces fall to the floor of the cage and dry out. Under these conditions no disease has been observed among *P. maculipennis* larvae even when reared under crowded conditions.

Climatic Conditions.

The most important climatic conditions which influence insect development are temperature, humidity and illumination. In their effects, these are generally reacting upon each other and thus only to a limited extent can they be considered separately.

Temperature.

Temperature is important because it determines the rate at which insects can be produced. The higher the temperature the shorter is the life-cycle, with the result that batches of insects may be reared with less labour in a shorter period of time. Thus, it is important to determine the highest temperature which satisfies the requirements of (1) relatively low mortality during development and (2) production of normal full-sized adults.

Effect of temperature on development of larvae and pupae.

Wigglesworth (1945) and Beament (1945) have shown that at a certain critical temperature the waterproofing wax layer of the insect is disturbed and there is rapid increase in transpiration through the cuticle. Therefore studies on rates of water loss in dry air at different temperatures were carried out and it was found that the

TABLE IV.

The effect of high temperatures on the development of *P. cochleariae.*

Temp. °C.	Per cent. relative humidity	No. of newly hatched larvae	Larvae		Pupae		Adults	
			Per cent. mortality after 7 days	Average wt. after 7 days (mg.)	Per cent. mortality prior to pupation	Average wt. (mg.)	Per cent. mortality prior to emergence	Average wt. (mg.)
30°C.	About 40	90	31	7·7*	38	6·5	42	5·4
	,, 100	90	19	7·6*	41	5·8	48	5·3
34°C.	,, 45	60	30	7·1*	72	5·6	100	—
	,, 100	60	82	3·5	100	—	—	—

*The majority of these were fully developed last-instar larvae.

critical temperature of a number of plant-feeding insects is low, 32–34°C. for larvae of *D. oleracea* and 30–32°C. for both larvae and adults of *P. cochleariae.* It would seem that temperatures in the region of the critical temperatures are deleterious to the insects and yet shade temperatures of over 38°C. are not unusual in the glasshouse in summer. Therefore experiments were carried out to determine the effect of such temperatures on insect development and Table IV shows the data obtained when larvae and pupae of *P. cochleariae* (the mustard beetle) were reared from hatching at constant temperatures of 30°C. (just below the critical temperature) and 34°C. (just above the critical temperature). At each temperature, the batches of larvae were reared at two different humidities in order to determine the significance of the increased water loss which occurs at the critical temperature. For pupation, moist peat was provided since even at low temperatures the pupae are desiccated at humidities below saturation.

At 30°C. the mortality during development to the adult stage, 42–48 per cent., was not excessive (see Table V) whilst humidity had little or no effect. At 34°C., and at the low humidity, larval mortality was no greater than at 30°C. Clearly the insect can obtain sufficient water from the plant to replace that lost as a result of the increased transpiration. However, a high mortality occurred at or just prior to the formation of the pupa and the remaining pupae all died at metamorphosis to the adult. At the high humidity all the larvae died before reaching the prepupal stage. Thus, at a high humidity a temperature of 34°C. is lethal for *P. cochleariae* larvae. It may be that normal development of the larvae occurs at the low humidity because a high rate of transpiration is possible and this may reduce the body temperature of the larvae to below the lethal level.

Under natural conditions *P. cochleariae* is seldom exposed to a temperature as high as 30°C. and Table V shows that this temperature is deleterious during the pupal, adult and egg stages, though for larval development it is almost as satisfactory as 24°C. It would seem that the use of the higher temperature is not justified in spite of the fact that it reduces the length of the larval and pupal stages from about 19 to about 13 days. The large size and high fecundity of adults reared at 24°C. suggest that this temperature is satisfactory for laboratory rearing.

TABLE V.

A comparison of 24°C. and 30°C. as temperatures for rearing the various stages of *P. cochleariae.*

No. of newly hatched larvae	Temperatures of				Per cent. mortality to adult stage	Mean wt. of newly emerged adults (mg.)	Total eggs /50 adults	Per cent. fertility eggs
	Larval stage	Pupal stage	Adult stage	Egg stage				
100	24	24	24	24	18	5·59	8,891	91
100	30	24	24	24	34	5·95	8,322	98
100	30	30	24	24	27	4·85	4,429	81
100	30	30	30	30	30	4·32	2,979*	38

*This figure was obtained from 49 adults.

Observations on the effect of temperature on larval development of *D. oleracea* were also made. During the winter months (December to February) a high mortality caused by disease has sometimes occurred among larvae reared at 24°C., but few diseased larvae have been observed among stocks reared at constant temperatures of 30°C. and 34°C. The incidence of disease appears to be related to high atmospheric humidity. At 24°C. it is not always possible, particularly during the winter months, to maintain a sufficiently dry atmosphere in cages containing quantities of food-plant.

By raising the temperature to 30 or 34°C., the saturation deficiency is markedly increased, condensation of water on foliage is prevented, frass dries out rapidly and disease is greatly reduced.

It would, therefore, appear that larval development of *D. oleracea* is more satisfactory at 30°C. and 34°C. than at 24°C. Experiments using batches of about 100 larvae showed that at 34°C. there was a high mortality at ecdysis from the prepupa to the pupa, 95 per cent. compared with 23 per cent. at 30°C. and 14 per cent. at 24°C. When a batch of 28 larvae reared at 34°C. was transferred to 30°C. at prepupal formation, the mortality at pupation was only 7 per cent. At 30°C. pupation occurs normally but pupae maintained at this temperature produce sterile adults (*cf.* Norris, 1933). However, in an experiment in which pupae were removed from 30°C. to a lower temperature (24°C.) directly after ecdysis from the prepupa, the resulting adults were normal and a batch of 1,580 eggs was laid that showed 78 per cent. fertility. It is of interest that 34°C. is lethal for eggs of *D. oleracea* whereas at 30°C. normal development occurs.

Effect of temperature on adults.

Experiments on the influence of temperature on adult behaviour were carried out with *P. cochleariae*. Batches of 25 newly emerged adults from larvae reared in a glasshouse were kept at constant temperatures of 12°C., 24°C. and 30°C. under 16 hours light per 24 hours (60-w. Tungsten) and with turnip as food-plant. Total numbers of eggs laid and the average lengths of life of the adults are shown in Table VI.

TABLE VI.

The effect of temperature on adults of *P. cochleariae*.

Temperature	Total eggs laid (25 adults)	Average length of life (days)
12°C.	4,248	124
24°C.	4,091	53
30°C.	3,456	44

The differences in numbers of eggs laid at different temperatures may not be significant because the proportions of the sexes in each batch of insects was not known. If it is assumed that there were equal numbers of each sex, the average number of eggs laid per female was 327 at 24°C. and 277 at 30°C. These figures compare favourably with those given by other investigators. Smith (1931) states that a female may lay 300-400 eggs while Hamnett (1944) gives the figure of approximately 100 eggs per female as a maximum. Clearly oviposition of *P. cochleariae* is satisfactory at constant temperatures using artificial illumination. The practical value of the higher temperatures for obtaining large numbers of eggs in a short period of time is obvious. It should be mentioned that a temperature of 30°C. during the adult stage is not deleterious to the fertility of the eggs (88–100 per cent. fertile) providing the eggs are removed to a lower temperature (24°C.) within about 12 hours of being laid. Even at a high humidity, mortalities greater than 60 per cent. have occurred among batches of eggs kept throughout development at 30°C.

Little information has been obtained on temperature requirements for oviposition of Lepidoptera. A temperature of about 16 to 21°C. has proved satisfactory for certain moths—*D. oleracea* (L.) (Table XIII), *Mamestra brassicae* (L.), *Plusia gamma* (L.), *Phlogophora meticulosa* (L.), *Agrotis segetum* (Schiff.) and *Mesographe forficalis* (L.), a conclusion which agrees with that of Kozhanchikov and others (1937) who found that fecundity of *A. segetum* was highest at 20°C. They also showed that high temperatures during the adult stage were detrimental. Only 20 per cent. of the adults copulated at 28°C. whilst no oviposition occurred at 32°C.

If insects are to be reared in a glasshouse, some attempt must be made to prevent sharp temperature fluctuations, while, in general, temperatures above the critical temperature must be avoided. However, glasshouse rearing is never satisfactory and for critical work, constant temperature rooms or cabinets are essential. The most suitable temperatures can then be chosen, standardisation of the rearing procedure becomes possible, and moreover, a knowledge of the rate of development of an insect at the required constant temperature enables pre-determination of when the stock will be available.

Humidity.

Disease in populations of plant-feeding insects is generally associated with high atmospheric humidity in the rearing cages. Such a condition is clearly indicated by the presence of wet faeces and by films or droplets of moisture adhering to the food plant. Thus, in rearing susceptible species, it is important that the humidity should be kept sufficiently low to ensure that the floor of the cage and the surface of the food-plant remain dry at all times.

Equipment for maintenance of low humidities is somewhat expensive but as shown previously, it is possible, at any rate in Britain, to produce relatively low humidities by raising the rearing temperature to about 30°C.

As distinct from its influence on disease, the direct effect of humidity on development of insects requires consideration. Ramsay and others (1938) have shown that the humidity above the leaf surface may be affected up to a height of over 1 cm. by the water vapour coming from the leaf. This is most noticeable when air movement is limited, a condition that is likely to occur in insect rearing cages. Thus, plant-feeding insects while on the plant are living in a micro-climate of high humidity. The extent of the micro-climate and the steepness of the humidity gradient are, of course, dependent upon external humidity but nevertheless, if the insect is enveloped or partly enveloped by a layer of humid air surrounding the leaves, it is unlikely to be affected markedly by changes in external atmospheric humidity. Low humidities are most likely to affect the insect when the temperature approaches the critical temperature and the normally efficient waterproofing mechanism of the insect cuticle begins to fail. It has been shown, however, that at about 45 per cent. relative humidity the larval stages of both *P. cochleariae* and of *D. oleracea* develop successfully at above the critical temperature (see Table IV). Clearly, desiccation caused by low humidities is not a problem for the feeding stages of these plant-feeding insects, providing food-plant is available.

The influence of humidity on the eggs and pupae requires investigation because, apart from certain exceptional cases, neither of these two stages in the life cycle is capable of replacing water lost by transpiration.

Effect of humidity on eggs.

The eggs of most insects (*Plusia gamma, Plutella maculipennis, Pieris brassicae* (L.)) discussed in the present paper are laid on the surface of the food-plant or in pits cut in the foliage by the female (*Phaedon cochleariae*, Plate XII, fig. 5). There is no doubt that they develop under conditions of high humidity and thus external changes in humidity are unlikely to influence the development of the egg, provided the food-plant remains alive. The adult of *P. cochleariae*, however, feeds actively on the egg-laying plant and by biting through leaf veins may cause areas of egg bearing foliage to wilt and die. Under these circumstances, eggs of this insect would be subject to influence by external humidity. Moreover, with some insects (*D. oleracea, Phlogophora meticulosa*), the most satisfactory method of obtaining eggs is to induce oviposition on muslin (Pl. XII, fig. 6) from which egg batches are cut out and stored until they hatch. It was felt that the effect of humidity on such eggs and on eggs of *P. cochleariae* should be determined. Newly laid eggs of *P. cochleariae* (on partly dried areas of foliage) and of *D. oleracea* (on muslin) were placed in small desiccators over phosphorus pentoxide and over water to give 0 per cent. and 100 per cent. relative humidities, and over saturated salt solutions to give a range of relative humidities between 19–96 per cent. The percentage of eggs that hatched at the different humidities at 24°C. are shown in Table VII.

TABLE VII.

The effect of relative humidity at 24°C. on viability of eggs of *D. oleracea* and *P. cochleariae.*

Species	No. of eggs / treatment	Percentage eggs hatching at different relative humidities							
		0%	19%	52%	64%	76%	86%	96%	100%
Phaedon cochleariae ...	60–100	0	0	0	4	34	90	86	89
Diataraxia oleracea ...	80–130	93	92	96	—	87	—	92	—

Clearly the eggs of *D. oleracea* are not affected by humidity (*cf.* Kozhanchikov, 1936) but those of *P. cochleariae* will not develop satisfactorily at relative humidities below about 86 per cent. Probably the egg absorbs water from the leaf during larval development (*cf.* Kerenski, 1930 ; Ludwig, 1932). The importance of a high humidity for eggs of *P. cochleariae* was confirmed with batches of 100–850 eggs, kept under normal rearing conditions at 24°C. and 60–70 per cent. relative humidity. On leaves which remained fresh throughout development, 95 per cent. of the eggs hatched. On those leaves which wilted and died as a result of excessive feeding by the adults, only 26 per cent. of eggs hatched, these having been laid on or near the main leaf veins which did not dry out to the same extent as the leaf lamina.

Effect of humidity on pupae.

As regards pupation, humidity both of the soil and of the atmosphere may have a marked effect on certain insects which normally pupate in the soil. Dry peat has proved to be a satisfactory pupation medium with *D. oleracea, M. brassicae* and *A. segetum* ; but peat, soil or sand, if they are damp cause a high mortality during ecdysis from the prepupa. Thus, at 24°C. a batch of 30 prepupae of *D. oleracea* showed 77 per cent. mortality at pupation in moist peat, while in dry peat at a relative humidity of 60–70 per cent. mortality at pupation was 13 per cent.

The direct effect of atmospheric humidity on pupation of *D. oleracea* was determined by placing prepupae in desiccators containing saturated salt solution to give a range of four relative humidities. The experiment was carried out at 24°C. Weight losses and mortalities at pupation are shown in Table VIII.

TABLE VIII.

The effect of atmospheric humidity at 24°C. on pupation of *D. oleracea.*

Relative humidity	No. of prepupae	Percentage wt. lost at pupation	Percentage mortality at pupation	Percentage wt. loss during pupal period (14 days)
20	31	18	26	29
50	29	12	14	22
75	23	9	17	17
100	22	8	5	3

The data suggest that a relative humidity approaching 100 per cent. is most suitable for pupation and for maintenance of pupae of *D. oleracea.* No information was obtained on the influence on the adults of humidity during the pupal period, but reference should be made to Kozhanchikov (1936a) who showed that with *A. segetum*, egg production was greatest when pupae were kept at 75 per cent. relative humidity (19°C.). Adults lived longest when pupae were kept at humidities of 75–95 per cent. but fecundity did not depend on longevity of the adult.

In contrast to *D. oleracea*, *P. cochleariae* does not pupate satisfactorily, even at a high atmospheric humidity, unless the pupation medium is moist and Table IX shows the effect of using moist and dry peat as a pupation medium for batches of 100 larvae. The normal rearing technique was adopted for the experiment (see Pl. XII, fig. 4).

TABLE IX.

Comparison of dry and moist peat as pupation media for *P. cochleariae*.

Temperature °C.	Relative humidity of atmosphere	Condition of pupation medium	Percentage mortality during prepupal and pupal stages	Average weight of adults at emergence (mg.)
24	About 70%	Dry	30	3·96
		Moist	8	5·11

A high proportion of adults which emerged from the pupae kept in dry peat had distorted or unexpanded wings. Clearly for *P. cochleariae* the pupation medium must be kept moist throughout the pupal period. It must not be too wet or the pupae are asphyxiated.

Illumination.

In previous descriptions of insect rearing procedures, illumination for some part of the day, generally 12 hours, has been recommended, though with regard to plant-feeding insects the value of light to the insect does not appear to have been determined. Accordingly simple experiments were carried out using artificial lighting during the rearing of a number of plant-feeding insects.

Effect of light on the larva.

Larvae of *P. cochleariae* were reared at 24°C. with artificial lighting for 12 hours per 24 hours and at three different intensities : (1) 1 ft.-candle from a 15-w. Tungsten lamp. (2) 25 ft.-candles from a 100-w. Tungsten lamp and (3) 220 ft.-candles from a 1,000-w. Tungsten lamp. (This lamp was cooled by immersion in a jacket of running water.) Batches of 20 of the resulting adults were kept under identical conditions (24°C. and under 16 hours light per 24 hours) and their fecundity determined. The total numbers of eggs laid are shown in Table X.

TABLE X.

The effect of light intensity during the larval period on the fecundity of adults of *P. cochleariae*.

Light intensity during larval period (ft.-candles)	No. of eggs laid by 20 adults
1	3,352
25	2,459
220	2,644

The difference in numbers of eggs laid may not be significant but it is clear that an abnormally low light intensity during the larval period is not deleterious to the adults.

Further observations were made with larvae of *P. cochleariae* and *D. oleracea* which were reared at 24°C. and under photoperiods varying from 0 to 24 hours per 24 hours at an intensity of approximately 25 ft.-candles. Detailed records were not kept but no differences in size or vigour of the developing larvae were observed. Also batches of *Plutella maculipennis* larvae were reared at 24°C. (1) under 16 hours illumination (25 ft.-candles per 24 hours) and (2) in darkness. The average number of eggs laid per female by batches (10♂♂+10♀♀) of the resulting adults kept under identical conditions at 24°C. are shown in Table XI.

TABLE XI.

The effect of light during the larval stage on the fecundity of adults of *P. maculipennis.*

Conditions of illumination during larval stage	Average no. of eggs laid per female
16 hours/24 hours	213
Darkness	195

The results show that complete absence of light during the larval stage has little or no effect on fecundity of the adults of *P. maculipennis.*

The above experiments suggest that illumination during the larval period is unlikely to affect the vigour of the resulting adults. Therefore careful standardisation of illumination is not necessary. However, it should be emphasised that the intensity and duration of light may affect the food-plant and thus influence the insects indirectly.

Effect of light on the adult.

Experiments were carried out in which batches of 20 (10♂♂+10♀♀) *P. maculipennis* adults were kept in a constant temperature room at 24°C. and under conditions of 0 (except for a few minutes per day), 8, 16 and 24 hours light (25 ft.-candles) per 24 hours. A further batch was kept in daylight (approximately 10 hours light per day) in the glasshouse under a widely fluctuating temperature (8–23°C. with a mean of approximately 16°C.). Honey solution was provided as food for the adults. The average numbers of eggs laid per female are shown in Table XII.

TABLE XII.

The effect on fecundity of light duration during the adult stage of *P. maculipennis.*

	Constant temperature (24°C.)				Fluctuating temperature (mean = 16°C.)
Period of light duration hours/24 hours	0	8	12	24	about 10 (daylight)
Average no of eggs/female ...	245	307	315	391	144

The table shows that light duration has little effect on fecundity though it was noted that all the adults kept in darkness were dead after 14 days while in the light treatments some were still alive after 20 days. The fertility was determined on batches of 400–700 eggs selected about 5 days after oviposition had begun. Percentage fertilities were: 92 per cent. for those laid by adults subjected to 0 hours light, 95 per cent. for those at 8 hours light, and 97 per cent. for those at 24 hours light.

The average number of eggs laid per female, even in darkness, is in excess of figures given by previous investigators : 70–90 (Smith, 1931) ; 172 at 20°C. (Hardy, 1938) ; 183 (Robertson, 1939). Other investigators record that the number of eggs laid by a single female may vary from 18 to 451 (see Robertson, 1939). Clearly, irrespective of conditions of illumination, oviposition of *P. maculipennis* at a constant temperature of 24°C. is highly satisfactory.

A similar experiment to the above was carried out with batches of adults (4♂♂+4♀♀) of *D. oleracea.* The control, using natural daylight, was kept in a laboratory by a window facing north. The average number of eggs laid per female and the fertility of batches of 150–400 eggs are shown in Table XIII.

TABLE XIII.

The effect on fecundity of light duration during the adult stage of *D. oleracea.*

	Constant temperature of 18°C.				Fluctuating temperature (18°C.±4°C.)
Period of light duration hours/24 hours	0	8	16	24	about 13 (daylight)
Average no. of eggs/female ...	583	1,134	964	741	1,121
Percentage fertility of eggs ...	76	82	80	67	94

It would appear that both darkness and continuous light are deleterious to the adults. At a constant temperature of 18°C. a photoperiod of eight hours' artificial light is probably as satisfactory as natural daylight. The figure for the number of eggs laid per female is in excess of that given by Lloyd (1920) who states that a female of *D. oleracea* will lay nearly 900 eggs.

Pieris brassicae apparently requires direct sunlight during the adult stage for both copulation and oviposition, and, so far, various forms of artificial illumination have proved unsuccessful. Thus, breeding of *P. brassicae* is not possible in winter and often somewhat uncertain during the summer.

With regard to *Phaedon cochleariae*, present evidence shows that complete absence of light during the adult period has a retarding effect on oviposition at 24°C. and may inhibit oviposition and induce hibernation at 12°C. This problem is now being studied in further detail by one of us (P. M. Smith).

Effect of light on diapause.

With some insects, light duration has a marked influence on diapause (Dickson & Saunders, 1945 ; Way and others, 1949). If larvae of *D. oleracea* reared at 24°C. are subjected to artificial light for 16–24 hours per 24 hours the resulting pupae are non-diapause, the length of the pupal stage being 15–30 days. With 14 hours' illumination per 24 hours, the resulting pupae enter diapause, which may last from about 50 to 200 days. The range of temperature over which light duration is the major controlling factor for diapause is limited and diapause is induced by low temperature (12°C.) during the larval stage and is partly prevented by high temperature (34°C.) irrespective of light duration (Way & Hopkins, 1950).

By using artificial light to give long photoperiods, continuous generations of *D. oleracea* may be reared. Moreover, preliminary studies have shown that light duration is similarly concerned in diapause of *P. brassicae* and *M. brassicae.*

A knowledge of the factors influencing diapause is essential for insect rearing and further studies on diapause in plant-feeding insects are now in progress. A knowledge of factors that prevent induction of diapause is important if the insects are required all the year round, but it is also valuable to be able to induce diapause. Thus, if an insect species is not required, diapause can be induced and the diapausing stage kept at a low temperature (2–3°C.) for up to one year and brought out when needed. This saves the labour of maintaining a stock when the insect species is not needed for experimental purposes.

General Rearing Methods.

As previously mentioned, an attempt has been made to develop rearing techniques which as far as possible can be standardised for a number of insect species. The degree of standardisation of breeding cages for adult oviposition, however, is limited mainly because conditions required for copulation and oviposition vary considerably

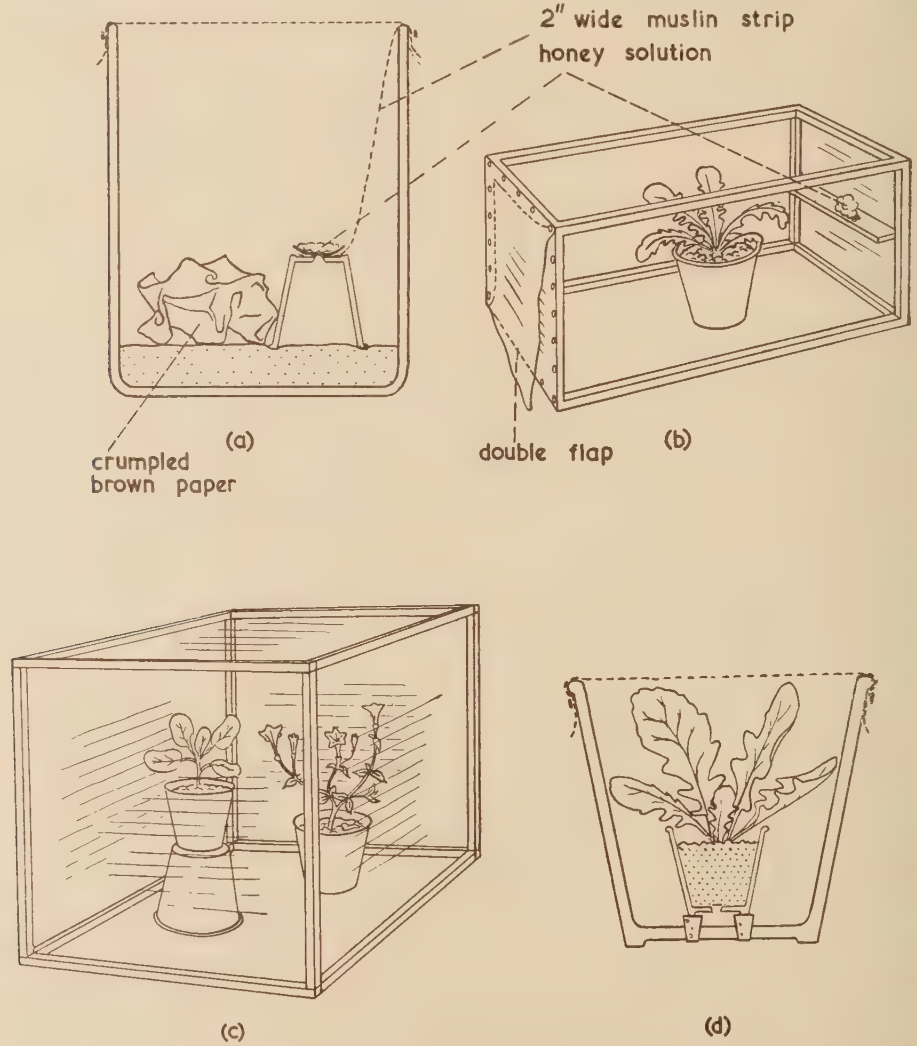

Fig. 1.—(*a*) Glass battery jar used for oviposition of certain Agrotids (*Phlogophora meticulosa, Diataraxia oleracea, Mamestra brassicae, Agrotis segetum*) ;
(*b*) Oviposition cage for *Plutella maculipennis, Mesographe forficalis, X. fluctuata* and *P. gamma* ;
(*c*) Large muslin covered oviposition cage for *Pieris brassicae* ;
d) 12 in. flower pot used as oviposition cage for *Phaedon cochleariae.*

according to the insect species. Four types of oviposition cages are shown in text figs. 1 *a*– *d*. Text fig. 1 *a* shows the type of cage which for the present work has proved suitable for Noctuid moths. It is similar to that used by Waters (1937) but the strip of muslin merely provides a foothold for the moths between the honey solution and the muslin cover to the cage. For oviposition, a potted plant may be placed in the cage but some species prefer to lay eggs on the muslin lid (Pl. XII, fig. 6). By replacing the muslin at daily intervals, batches of eggs of known age are obtained. For ovicidal tests the eggs may be readily removed after damping the muslin with water.

The cage shown in text fig. 1 *b* is suitable for certain moths (*Plutella maculipennis*, *Mesographe forficalis*, *Xanthoroë fluctuata* and *Plusia gamma*) which may become active when disturbed. Eggs are laid on the potted plant which can be removed or replaced from the darkened end of the cage without allowing the moths to escape.

Oviposition cages for cabbage butterflies (fig. 1 *c*) are kept outdoors in the sunlight. They are covered with 1/2 cm. mesh net and should be as large as possible to allow for some form of mating flight.

For *P. cochleariae* a small oviposition cage is required, a 12-in. flower pot being suitable (fig. 1 *d*).

Small insects and early instars of larger insects are reared on potted plants. Plants or individual leaves bearing eggs or developing larvae are enclosed in muslin bags (Pl. XII, figs. 3–4). When the foliage has been almost consumed a fresh leaf is inserted into the bag. The insects move on to this leaf and during the following day the remnants of the old leaf are removed. In this way handling of the young larvae is largely eliminated. Aeration is good and the insect faeces dry out rapidly providing the foliage is not crowded into the muslin bag. Moreover, the young larvae are kept in close contact with the food-plant and do not stray. The bagging method has proved satisfactory for most of the insect species discussed in the present paper and is less tedious and involves less handling of the insects than methods previously described (see Waters, 1943).

Larvae of the larger insect species are transferred to cages (Pl. XII, fig. 2) at about the third instar and fed on cut foliage until they pupate. Each cage contains two bottles of foliage which are replaced alternately. All the larvae are placed on the foliage of one bottle. After 2–3 days this foliage is almost completely consumed and most of the larvae have moved on to the foliage in the second bottle. Relatively few larvae require to be transferred by hand. Fresh foliage is then added to the first bottle which is replaced in the cage.

Rearing Methods for Individual Species.

Few of the species which have been studied possess all of the desirable properties mentioned in the introduction. The most successful results have been obtained with Lepidoptera, mainly because this order includes a large number of leaf-eating species from which to choose.

In Britain there are no plant-feeding Orthoptera of economic importance and accordingly the African migratory locust, *Locusta migratoria* (L.) was chosen. The rearing technique suggested by the Anti-Locust Research Centre (London) has proved satisfactory. No further reference need be made to the rearing of Orthoptera which is discussed by Waters (1943).

Only a few species of leaf-eating Hymenoptera and Coleoptera have been obtained and of these, *Phaedon cochleariae* has proved satisfactory.

The following species have been tested for laboratory rearing.

LEPIDOPTERA.

1. Plutellidae. Diamond Back Moth, *Plutella maculipennis* (Curt.).
2. Pyraustidae. Garden Pebble Moth, *Mesographe* (*Pionea*) *forficalis* (L.).
3. Geometridae. Garden Carpet Moth, *Xanthorhoë fluctuata* (L.).
4. Agrotidae, Plusiinae. Silver Y Moth, *Plusia gamma* (L.).
5. ,, Hadeninae. Tomato Moth, *Diataraxia oleracea* (L.).
6. ,, ,, Cabbage Moth, *Mamestra brassicae* (L.).
7. ,, Acronyctinae. Angle Shades Moth, *Phlogophora meticulosa* (L.).
8. ,, Agrotinae. Turnip Moth, *Agrotis segetum* (Schiff.) and Large Yellow Underwing, *Triphaena pronuba* (L.).
9. Lymantriidae. Gypsy Moth, *Lymantria dispar* (L.).
10. Sphingidae. Privet Hawk Moth, *Sphinx ligustri* L.
11. Pieridae. Large Cabbage White Butterfly, *Pieris brassicae* (L.).

COLEOPTERA.

12. Chrysomelidae. Mustard Beetle, *Phaedon cochleariae* (F.).
13. Silphidae. Beet Carrion Beetle, *Aclypea opaca* (L.).
14. Crioceridae. Cereal Leaf Beetle, *Lema melanopa* (L.) and Asparagus Beetle, *Crioceris asparagi* (L.).

HYMENOPTERA.

15. Symphyta. Turnip Sawfly, *Athalia colibri* (Christ).

Details of the rearing methods for each species are as follows :—

Lepidoptera.

1. *Plutella maculipennis.*

This species is world wide in distribution and the fact that it can be bred satisfactorily at temperatures from 10° to 40°C. (Hardy, 1938) suggests that it is unlikely to be difficult to rear in the laboratory. Moreover, there is no definite diapause stage and thus it can be reared continuously providing the temperature is above 10°C. In winter the species may hibernate either as an adult or as a pupa (Smith, 1931 ; Hardy, 1938). The larva attacks many cruciferous weeds and all cultivated crucifers, particularly turnip and swede.

About 30 individuals were reared in a constant temperature room at 24°C. and the average lengths of various stages recorded as follows : egg stage, 3 days ; larval stage (reared on cabbage), 11 days ; pupal stage, 6 days. Thus it is easy to rear about ten generations of this species in one year.

In the field the moth is very susceptible to damp conditions (Gunn, 1917 ; Smith, 1931) when entomogenous fungi (Kanervo, 1936) and what appears to be a polyhedrosis may be destructive to the larvae. Similarly in the laboratory, disease may occur among larvae unless humid conditions are avoided. A rearing method has been described by Swingle (1935) and Swingle and others (1941). This has not been adopted because it would seem that conditions of high humidity cannot always be avoided and moreover the use of cut foliage may necessitate handling of the larvae.

The following is the present rearing technique. The oviposition cage (fig. 1 *b*) will hold about 200 adults. A constant temperature of 24°C. and artificial lighting of 25 ft.-candles for more than eight hours per day is satisfactory (see Table XII). Turnip is more suitable than cabbage for oviposition and also for development of the first-instar larvae ; a well-developed turnip plant in a 5-in. pot and a small dish containing cotton wool soaked in honey solution are replaced in the oviposition cage at daily intervals.

The turnip plant provides sufficient food for development of the first two larval instars. The turnip leaves bearing the second- or third-instar larvae are then gently removed and laid on leaves of a " bolted " cabbage plant in a 7-in. pot or other variety of *Brassica oleracea* with open growth on which they complete their development (Pl. XII, fig. 1). Various brassicas have been used as host-plants for the third- and fourth-instar larvae including spring, summer and winter cabbages, savoy, broccoli, brussels sprouts, kale, and colewort. The larvae appear to feed equally well on all types providing the foliage is not old and coarse. As mentioned before, hearted brassica plants are not satisfactory because they promote conditions which encourage disease. In the glasshouse, the plants are enclosed in 30 × 30 × 30-in. muslin cages which prevent access of parasites.

The larvae pupate on the food-plant. The cocoons are removed and batches of about 200 are put into each oviposition cage.

P. maculipennis is one of the few leaf-eating species which satisfy all the requirements of a laboratory test subject. A vigorous stock has been reared continuously in this laboratory for the past seven years.

2. *Mesographe* (*Pionea*) *forficalis*.

This species is a pest of crucifers. It has not as yet been reared in large numbers under laboratory conditions but it has a number of useful qualities as a test subject.

The cage shown in fig. 1 *b* is used for oviposition, honey solution being provided as food for the adults, and a potted cabbage or turnip plant for oviposition. A temperature of about 18°C. is suitable, while direct sunlight must be avoided. The eggs are relatively large and are laid in batches on both surfaces of the leaves.

As soon as the eggs begin to hatch the plant is enclosed in a muslin bag (Pl. XII, fig. 3). The later instars are fed on cut brassica foliage (Pl. XII, fig. 2). Disease has not been observed except when larvae were reared under humid conditions (*cf. Plutella maculipennis*).

At a constant temperature of 24°C. the egg stage lasts about six days and the larval stage 20 days. The fully developed larva leaves the plant and spins a cocoon in dry peat at the bottom of the cage. Diapause may occur as a fully developed larva in the cocoon, but otherwise there is a short prepupal stage and a pupal period of about 14 days at 24°C.

The main disadvantage of this species is that in all batches reared so far 62–100 per cent. of larvae have gone into diapause. This occurs even with a photoperiod of 16 hours per 24 hours at 30°C. Results so far have shown that subjection for a period of time to a low temperature is not required to break diapause, since a batch of diapausing larvae kept at a constant temperature may begin to pupate and emerge as adults about one month after the beginning of diapause. Pupation of individual larvae may continue at intervals over the next six months. Diapause was not broken by opening the cocoon and inducing respinning (*cf.* Theron, 1943).

In the absence of knowledge of the factors affecting diapause, the only certain method of rearing this species throughout the year is to maintain a large stock of diapausing larvae, of which a sufficient proportion are always pupating to ensure a continuous supply of adults of both sexes.

3. *Xanthorhoë fluctuata*.

Larvae are occasionally found on cultivated crucifers. Although not a pest it has proved suitable for rearing in the laboratory.

The rearing technique is the same as that previously described for *M. forficalis*. A potted turnip is used for oviposition though some eggs may be laid on any rough surface in the cage (*cf. Plusia gamma*). The young larvae feed on the turnip foliage and are later transferred to cut foliage of brussels sprouts or cabbage (Pl. XII, fig. 2).

Pupation occurs in dry peat at the bottom of the cage. Generally over 80 per cent. of pupae produced in the glasshouse during the winter months are of the diapause type, and at 18°–24°C. diapause may last for one to six months. Thus from a batch of diapausing pupae, adults may emerge at intervals over a period of about six months (*cf. D. oleracea,* Way & Hopkins, 1950). Low temperatures are not required to break the diapause, but if diapausing pupae are kept at 2°–3°C. for a period of four to five months and then brought out to 24°C. all adults emerge within a period of two to four weeks. Larvae reared in the glasshouse in the summer months (May to July) have resulted in pupae, of which at least 80 per cent. have been of the non-diapause type. Thus diapause appears similar to that of *D. oleracea* though unfortunately the rearing of this species was discontinued before the importance of photoperiodism as a factor affecting diapause of *D. oleracea* was realised. Consequently, it is not known whether artificial production of long photoperiods will enable *X. fluctuata* to be reared continuously throughout the year without difficulty.

4. *Plusia gamma.*

This species feeds on a wide variety of food-plants and develops satisfactorily on brassicas. Several individuals were reared at 24°C. with turnip and cabbage as larval food-plants and the average lengths of various stages were recorded as follows : egg stage, five days ; larval stage, twelve days ; pupal stage, 8 days. Thus at 24°C., eight to ten generations may be reared per year.

In oviposition cages (fig. 1 *b*) honey solution is provided as food for the adults and a potted turnip for oviposition. A temperature of about 18°C. is suitable whilst direct sunlight must be avoided. The adults will not lay satisfactorily on smooth leaved plants such as the cabbage, but they sometimes lay on the muslin walls of the cage which as far as possible should be replaced by perforated zinc.

Young larvae are reared in muslin bags (Pl. XII, fig. 3) and later are transferred to cut foliage of brussels spouts or other variety of brassica (Pl. XII, fig. 2). The larva generally pupates on the food-plant or on the muslin walls of the cage. A temperature of 24°C. is suitable for larval development. Development at 30°C. has not been studied.

Although it may overwinter as an adult (Fisher, 1938), *P. gamma* shows no definite diapause stage and can be reared continuously throughout the year providing the temperature is maintained above about 17°C.

The larvae appear to be resistant to disease providing the humidity is maintained at a sufficiently low level. A virulent polyhedrosis has been observed when larvae were reared under humid and crowded conditions. Such conditions can always be avoided and should never be allowed to develop in cultures of any plant-feeding species.

A minor disadvantage of this species is that the eggs are laid singly and somewhat promiscuously by comparison with other Agrotids. In other respects, particularly with regard to absence of diapause, *P. gamma* appears well suited for laboratory rearing.

5. *Diataraxia oleracea.*

The larvae feed on various cultivated plants including brassicas. They have caused much damage to tomatos in glasshouses (Lloyd, 1920).

Thirty individuals were reared in a constant temperature cabinet at 24°C. and the average lengths of the various stages in the life-cycle were recorded as follows : egg, five days ; larva, 25 days ; pupa (non-diapause), 15–21 days.

About 20 adults are placed in each oviposition cage (fig. 1 *a*). Fresh honey solution is provided daily and the cage is washed out about twice a week. Direct sunlight is harmful and a window facing north at a temperature of about 18°C. is suitable for oviposition. A constant temperature of 18°C. with artificial lighting of 25 ft.-candles for eight hours per 24 hours is also satisfactory (see Table XIII).

The eggs are laid in batches on the muslin cover of the cages (Pl. XII, fig. 6). Small areas of muslin bearing the egg batches are cut out and just prior to hatching are enclosed in muslin bags with leaves of potted dock (*Rumex* sp.) or brassica (Pl. XII, fig. 3). Dock is preferred by first-instar larvae unless the brassica foliage is young and tender. At about the third instar the larvae are transferred to larger cages and fed on cut brassica foliage (Pl. XII, fig. 2). Pupation occurs in dry peat on the floor of the cage. The peat must not be wet.

Under damp conditions the larvae are susceptible to disease, but as shown previously, rearing at 30°C. is not detrimental to the larvae although, if pupae are kept at this temperature, the resulting adults are sterile. A temperature of 24°C. and a relative humidity of over 75 per cent. is satisfactory for pupal development.

Diapause in *D. oleracea* has already been discussed and it has been shown that if larvae reared at 24°–30°C. are subjected to artificial light for at least 16 hours per 24 hours the resulting pupae are non-diapause. Thus, *D. oleracea* may be reared continuously throughout the year.

From an original stock of 15 individuals, *D. oleracea* has been reared in large numbers throughout the last six years and has been proved very satisfactory both for laboratory rearing and as a test subject.

6. *Mamestra brassicae.*

Larvae of this species are well known as pests of brassicas. About 30 individuals were reared in a constant temperature cabinet at 24°C. ; the average lengths of various stages in the life-cycle were : egg, six days ; larva, 27 days ; pupa (non-diapause), about 20 days.

The rearing technique is essentially similar to that used for *D. oleracea.* Egg laying occurs readily on potted cabbage plants placed in the adult breeding cage (fig. 1 *a*) although batches of eggs may be laid on the muslin and also on the glass walls of the cage, particularly if these are not clean. The young larvae are reared on potted brassica plants in muslin bags (Pl. XII, fig. 3) and the later instars on cut brassica foliage (Pl. XII, fig. 2). The larvae are not susceptible to disease except under humid or crowded conditions.

Diapause occurs during the pupal stage but differs somewhat from that of *D. oleracea* because it is not broken unless the pupae are kept for about four months at a low temperature (2°–3°C.). Diapause pupae kept at a constant temperature of 24°C. may live for six months to one year, but eventually die without metamorphosing to the adult. The factors affecting diapause are somewhat obscure. The pupae resulting from larvae reared at 24°C. and under winter daylight are all of the diapause type, but if the larvae are reared under 16 hours artificial light per 24 hours approximately 80 per cent. of the resulting pupae are non-diapause. However, the second generation pupae are practically all of the diapause type regardless of a 16 hours photoperiod during the larval stage. Thus it has been possible to rear only two consecutive generations of this species. Nevertheless, a stock of diapause pupae may be kept at 2°–3°C. for up to one year and these can be used to rear the insect at any time of the year that it is required.

Although this species can be reared successfully in the laboratory, the larvae possess a serious disadvantage as test subjects for studies on insecticides. As in the case of other Lepidoptera, larvae of *M. brassicae* treated with certain insecticides, such as DDT, may remain affected for a period of several days before either recovering or dying. During this period, a high proportion of the larvae of this species may contract disease and die, thus making it impossible to assess the direct effect of the insecticide.

7. *Phlogophora meticulosa.*

Parr and Speyer (1941) report that the larvae may be pests of a wide variety of cultivated plants.

P. meticulosa is reared using the same technique as that described for *D. oleracea*. The larvae, which develop well on any brassicas, appear to be more resistant to disease than those of *D. oleracea* and *M. brassicae* and they have proved admirable test subjects for studies on insecticides.

There is apparently no diapause stage although overwintering may occur as a pupa (Parr & Speyer, 1941) or as a partly developed larva. As regards ease of rearing, the eggs are laid singly and not in batches—a minor disadvantage. A more serious disadvantage is that on the three occasions that individuals were obtained, either second or third generation females have produced only sterile eggs. Experiments with *D. oleracea* and *P. cochleariae* have shown that temperature during the pupal period may have a considerable effect on adult fecundity, and data given by Parr and Speyer (1941) indicate that a relatively low temperature is required for normal development of the pupa of *P. meticulosa*. It is hoped to study this problem in further detail since this insect is in many respects a valuable test subject.

8. *Agrotis segetum* and *Triphaena pronuba.*

Detailed studies have been carried out with *A. segetum* in an attempt to rear this important cutworm in the laboratory. It overwinters as a last-instar larva which will become active and feed normally whenever the temperature is sufficiently high, thus enabling continuous generations to be reared. After rearing six continuous generations in the laboratory, however, adult fecundity began to deteriorate. Moreover, the larvae are susceptible to disease and are cannibalistic. They also become hyperexcitable on handling or disturbance. Therefore, it was concluded that *A. segetum* was unsuitable for laboratory rearing. Experiments with *T. pronuba* showed that the larva has the same disadvantages as those of *A. segetum*.

9. *Lymantria dispar.*

In the United States the larvae are serious pests of deciduous trees, but in Britain *L. dispar* is extinct or very rare.

As regards laboratory rearing, there are two disadvantages : (1) the food (foliage of deciduous trees) is not available in winter and (2) diapause occurs in the egg stage after only one generation. The diapause in the egg is broken after about four months at 2–3°C. Eggs may be kept at this temperature for a further eight months and brought into a higher temperature whenever larvae are required so that by maintaining a large stock of eggs at 2°C., larvae may be obtained and reared during any part of the year when food is available, *i.e.*, about April–October.

In many respects *L. dispar* possesses admirable properties for laboratory rearing. The adults do not feed and no difficulties have been experienced with copulation and oviposition. The adults may be enclosed in any dish or cage containing crumpled paper on which large batches of eggs are laid. A temperature of about 18°C. is suitable and radiant heat from sunlight should be avoided.

Until about the third instar, the larvae are reared on small branches of either hawthorn, lime, poplar or other available deciduous tree enclosed in muslin bags. They are then transferred to cages containing bottles of cut foliage (Pl. XII, fig. 2).

Pupation occurs on the food-plant or on the cage walls. The sexes are readily distinguishable in both pupal and adult stages—the female being much the larger.

Although it is available for only six months of the year, this species is relatively simple to rear in large numbers. A further advantage is that the food, when available, may be readily obtained from the hedgerows and does not require to be grown specially.

10. *Sphinx ligustri.*

This is not a pest but is suitable for studies requiring relatively large species. Its main advantages are that the food-plant is readily obtainable and the larva is apparently resistant to disease.

The adults require a relatively large breeding cage of the type used for *Pieris brassicae* (fig. 1 *c*). Direct sunlight must be avoided and a room with a window facing north at a temperature of about 18°C. has proved suitable. A slowly moving fan is used to draw a gentle air current across the cage. This appears to be beneficial but direct proof has not been obtained. Crumpled paper should be provided for the adults to rest in during the day. Cotton wool soaked in honey solution has also been provided although it is not known whether the adults require either food or water. Under these conditions oviposition has always been satisfactory. Eggs are laid indiscriminately over the walls and floor of the cage even when branches of larva food are provided. The eggs are picked up with a damp paint brush and may be stored at 24°C. until just prior to hatching. They are then transferred to muslin bags enclosing branches of cut privet.

A temperature of 24°C. is satisfactory for larval development. The technique of rearing the larvae is the same as for other large Lepidoptera. At about the third instar, the larvae are transferred from muslin bags to cages (Pl. XII, fig. 2) in which development is completed. Pupation occurs in dry peat on the floor of the cage.

Diapause occurs in the pupal stage and appears to be obligate since all attempts to prevent diapause and rear even two continuous generations have hitherto been unsuccessful. Diapause is not broken unless the pupae are kept at a low temperature (2–5°C.) for about six months. Pupae may be kept at this temperature for a further 18 months and at any time during this period may be removed to a warm temperature to provide fertile adults.

Apart from the problem of diapause, no difficulty has been encountered in rearing this species ; the larva is sluggish and not disturbed by handling and it should prove valuable as a test subject whenever a large insect is required.

11. *Pieris brassicae.*

Larvae of this species are well known as pests of brassicas.

About 30 individuals were reared in a constant temperature cabinet at 24°C. and the average lengths of various stages in the life-cycle recorded were : egg, five days ; larva, 17 days ; pupa (non-diapause) seven days. Thus the rate of development of this butterfly is more rapid than that of moths of similar size (*D. oleracea, M. brassicae*).

A relatively large wide-mesh muslin cage is suitable for breeding the adults. A potted cabbage plant is supplied for oviposition and a flowering petunia plant with flowers containing drops of honey solution is suitable for providing the necessary sugar and water.

Copulation and oviposition are unsatisfactory unless the insects are exposed to direct sunlight at a temperature above about 20°C. It has been observed that, even in a sunlit glasshouse, the males tend to be inactive but begin to copulate as soon as the breeding cage is placed outdoors. Perhaps ultra violet light (which is largely absorbed by ordinary glass) is important for inducing copulation. It is known that *P. rapae* will copulate in the light of a 60-w. Tungsten bulb but attempts to induce copulation of *P. brassicae* by using various artificial lighting systems (tubular fluorescent, 400-w. mercury fluorescent and 1,000 Tungsten lamps) have been unsuccessful. No studies have been made using an ultra violet lamp.

The rearing of *P. brassicae* is not possible at present during the winter and at all times is dependent upon the presence of sunlight. In one year suitable conditions prevailed and five continuous generations of *P. brassicae* were reared between April and November but this was exceptional.

Apart from inducing oviposition and controlling parasites, no further difficulty has been experienced in rearing. The young larvae do not stray and are reared on potted plants (Pl. XII, fig. 1) under muslin cages. The later instars may also be reared on potted plants or in cages containing cut foliage (Pl. XII, fig. 2). Disease does not occur among the larvae if damp conditions are avoided. Pupation occurs on the walls of the cage.

Diapause occurs in the pupal stage and, as in the case of *D. oleracea*, is dependent on the photoperiod during the larval stage. Thus at 24°C., using artificial light, a photoperiod of over 16 hours per 24 hours prevents onset of diapause. Otherwise under natural daylight a high percentage of larvae reared in spring and autumn result in diapause pupae. Diapause may be broken by keeping the pupae four to five months at a temperature of 2–5°C. and then removing to a higher temperature. However, the pupae may be stored at the lower temperature for a further eight months and removed whenever adults are required. Diapause is not broken unless the pupae are subjected to cold conditions (*cf. M. brassicae*).

Further attempts will be made to determine the conditions required for adult copulation and oviposition of *P. brassicae*, but, at present, successful rearing is dependent upon conditions of temperature and sunlight and perhaps other factors which influence breeding of the adults. Rearing of *P. brassicae* in large numbers is not possible in winter and somewhat uncertain even in summer.

Coleoptera.

12. *Phaedon cochleariae.*

The adults and larvae are pests of mustard and other brassicas, and watercress.

About 30 individuals were reared in a constant temperature cabinet at 24°C. and the average lengths of the various stages in the life-cycle were recorded as egg, eight days ; larva, 16 days ; pupa, six days. The adults may live two to three months at this temperature (see Table VI).

About 100 adults are placed in each breeding cage (fig. 1 *d*) together with a well-developed turnip plant in a 5-in. pot. As mentioned before, feeding by the adults is liable to cause areas of egg-bearing foliage to wilt and die with the result that the eggs fail to develop but at 24°C., about 100 adults seldom cause death of any part of the foliage providing the turnip plant is replaced daily. White mustard foliage (*Brassica alba*) is unsuitable for oviposition because of its sensitivity to feeding damage.

Individual turnip plants are enclosed in muslin bags just prior to hatching of the eggs (Pl. XII, fig. 4 *a*). When most of the turnip foliage has been consumed by the young larvae, a white mustard plant is inserted into the muslin bag and the remnant of the turnip foliage removed during the following day. White mustard is used as far as possible because it is a more satisfactory food-plant than turnip (see Table I). Moreover, growth of white mustard under winter conditions of daylight is more satisfactory than that of turnip. Cabbage and kale appear to be unsatisfactory food-plants for the larva (Table I).

When the larvae are fully developed, a crystallising dish containing moist peat is inserted into the top of the muslin bag (Pl. XII, fig. 4 *b*). Pupation occurs in the peat : the pupae, being delicate, must not be disturbed.

Adults are allowed to emerge into a cage containing turnip or mustard plants. After feeding voraciously for about a week they begin to lay eggs and are then transferred to the breeding cages (fig. 1 *d*).

A constant temperature of 24°C. is satisfactory for development of all stages. A temperature of 30°C. increases the rate of development, but adults resulting from pupae reared at this temperature are undersized (Table V). Furthermore, although adults kept at 30°C. lay fertile eggs, there is a high mortality during the egg stage unless the eggs are removed to a lower temperature (24°C.) after being laid. At 24°C. *P. cochleariae* shows no sign of seasonal rhythm because hibernation of the adult occurs only at a temperature lower than about 12°C. Both temperature and light are concerned in the induction of hibernation but the optimum conditions have not yet been determined. It is known, however, that hibernating adults may be kept at 2–5°C. for at least six months. If they are removed at any time during this period and the temperature gradually increased over a period of about a week to 24°C. egg laying begins in about two to three weeks.

Present evidence suggests that under normal rearing conditions humidity has little influence on the feeding stages of *P. cochleariae*. The larvae and adults are not susceptible to disease even at 100 per cent. relative humidity. Low humidities prevent development of eggs laid on foliage which has wilted and died but as mentioned before, this condition can be avoided. With regard to the pupae, if the pupation medium (moist peat) is allowed to dry out, the resulting adults are seriously affected (Table IX).

Light appears to have no effect on larval development, but its absence during the adult stage may retard oviposition at 24°C.

P. cochleariae may be reared in the laboratory without difficulty throughout the year. This species is considered to be a satisfactory and useful test subject.

13. *Aclypea opaca.*

On two occasions batches of larvae and adults have been obtained. These were kept in a cage, the floor of which was covered by about 3 ins. of soil in which young plants of sugar beet were growing. On the first occasion the adults killed and partly consumed the larvae. No eggs were found. On the second occasion a batch of 30 larvae were reared in one cage and eight pupae obtained. Two adults emerged and immediately consumed the remaining pupae. Again no eggs were found.

Even if adults can be induced to breed in the laboratory it seems certain that individual rearing would be necessary to prevent cannibalism. This is impracticable for rearing relatively large numbers and thus it was concluded that *A. opaca* is unsuitable for laboratory rearing.

14. *Lema melanopa* and *Crioceris asparagi.*

Small numbers were reared in the laboratory but the cultures were allowed to die out largely through lack of attention. In the light of the present knowledge on factors affecting development of *P. cochleariae* it seems probable that *L. melanopa,* which is similar in habit to *P. cochleariae,* would be a particularly suitable species for laboratory rearing. The food plant (foliage of oats, barley and wheat) can be grown in quantity throughout the year.

Hymenoptera.

15. *Athalia colibri.*

Attempts were made to rear this sawfly from a small stock of larvae. These were kept at 24°C. with artificial light for 16 hours per 24 hours but a high proportion went into diapause. Owing to the widely spaced emergence of the adults and the increasing proportion of larvae going into diapause in successive generations, few pairings were obtained and the size of the stock had not increased even after four generations.

Discussion.

In order to rear plant-feeding insects satisfactorily, some knowledge was required of factors influencing their development. The food-plant, temperature, humidity and illumination appeared to be the most important and consequently experiments were carried out to determine their influence on development of leaf-feeding insects. Two representative species, *Diataraxia oleracea* (Lepidoptera) and *Phaedon cochleariae* (Coleoptera) were chosen for special study.

The two species differ in their responses to humidity (Tables VII–IX) but not in their optimum temperature range. Present evidence suggests that the temperature and humidity optima for rearing the larvae of other leaf-eating Lepidoptera and Coleoptera are similar to those of *D. oleracea* and *P. cochleariae* respectively. As regards choice of food-plant, each species requires individual study. It is also clear that no general principles can be stated regarding either factors influencing diapause or mode of diapause of plant-feeding insects since these may differ even in nearly related species. This problem is of considerable importance in the rearing of plant-feeding insects and further studies on factors influencing diapause in the various insects are now in progress.

With regard to ease of rearing, it should be mentioned that even the most satisfactory plant-feeding insects, because of their mode of life, require more labour and more frequent and careful attention than the widely used stored products and household insects. Owing to specificity of insecticidal action, however, the data obtained in tests using stored products insects is not applicable to insects of agricultural importance.

Summary.

A number of leaf-eating insects have been studied with the object of selecting species suitable for rearing in the laboratory.

Four species have proved satisfactory for large-scale rearing throughout the year. These are *Plutella maculipennis, Plusia gamma, Diataraxia oleracea* and *Phaedon cochleariae.* A number of species possess certain disadvantages : (1) apparently obligate diapause (*Mamestra brassicae, Sphinx ligustri*). (2) The same as (1) with the additional disadvantage of lack of food-plant in winter (*Lymantria dispar*). (3) Uncertain copulation and oviposition (*Pieris brassicae*). When available, these species may be reared satisfactorily in large numbers.

Mesographe forficalis, Xanthorhoë fluctuata, Lema melanopa and *Crioceris asparagi* have been reared in relatively small numbers. They possess certain disadvantages but should be satisfactory for large scale rearing at any rate during some part of the year.

At present laboratory rearing of *Phlogophora meticulosa, Agrotis segetum, Triphaena pronuba, Aclypea opaca* and *Athalia colibri* is considered impracticable. A number of leaf-eating species, particularly Hymenoptera, which appear to possess suitable qualities for laboratory rearing, have not yet been obtained and studied in the laboratory.

Particular attention has been paid to factors influencing development of leaf-feeding insects with the object of determining the conditions under which healthy stocks of uniform resistance can be maintained. The results of experiments on the effect of food-plant, temperature, humidity and illumination are described. Studies on the factors influencing diapause in the various species are mentioned. Descriptions are given of the rearing techniques that have been developed.

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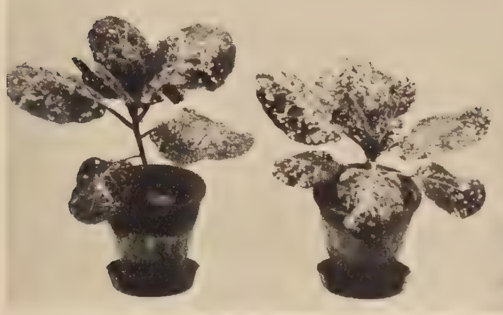


FIG. 1.

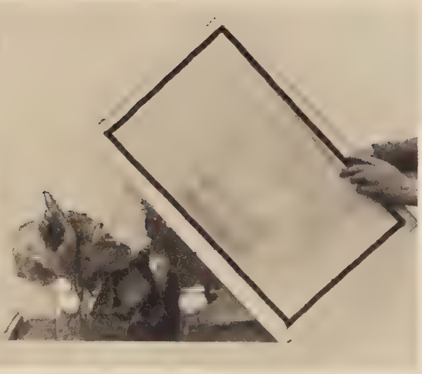
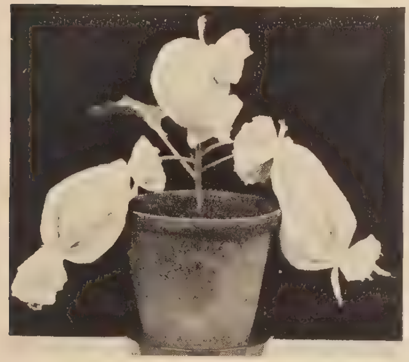


FIG. 2.



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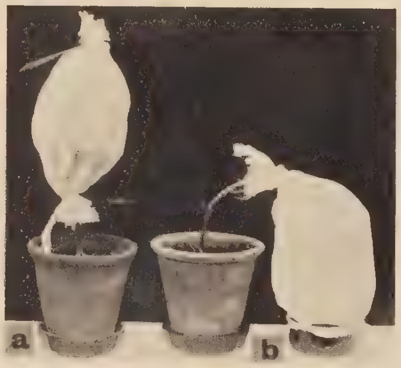

FIG. 4.

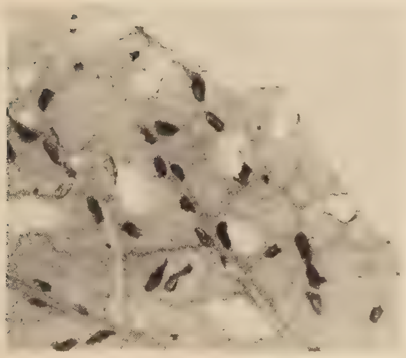

FIG. 5.

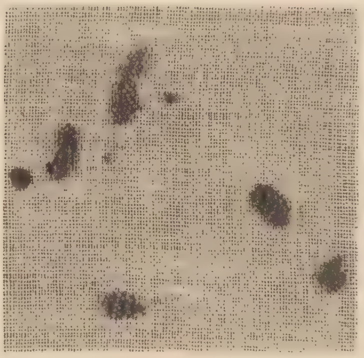

FIG. 6.

FIG. 1. Cabbage plants with open growth used for rearing the late instar larvae of *P. maculipennis* and the early instar larvae of *P. brassicae*.

FIG. 2. Cage containing cut foliage for rearing the late instar larvae of various Lepidoptera.

FIG. 3. " Bagged " leaves of cabbage on which the young larvae of various Lepidoptera are reared.

FIG. 4. (*a*) A " bagged " mustard plant for rearing larvae of *P. cochleariae*.
(*b*) A " bagged " mustard plant with attached crystallising dish containing moist peat for pupation of *P. cochleariae*.

FIG. 5. Eggs of *P. cochleariae* on the undersurface of a turnip leaf.

FIG. 6. Batches of eggs of *D. oleracea* laid on part of the muslin cover of an oviposition cage.

THE BIONOMICS OF AN AFRICAN *MEGARHINUS* (DIPT., CULICIDAE) AND ITS POSSIBLE USE IN BIOLOGICAL CONTROL.

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The mosquitos with which this paper is concerned have sometimes been described as "elephant" mosquitos, probably on account of their large size. For many years, they have been known to have a larva which is predatory on other mosquito larvae. Neave (1940) has shown that the generic name *Megarhinus* Robineau-Desvoidy is preoccupied by *Megarhinus* Rafinesque, a manuscript name validated under Opinion 4 of the International Rules of Zoological Nomenclature (see Stone, 1948). The name *Toxorhynchites* Theobald ought therefore to be substituted, but for convenience the more familiar name is retained in the present paper.

The adults of the genus are flower feeders and do not suck blood. Observations on the taking of nectar from flowers by them have been published by Knab (1911), Urich (1913) and Senior White (1920). Practical interest has been aroused by attempts to use these mosquitos in the biological control of those *Aëdes* mosquitos which carry the parasites (*Wuchereria bancrofti*) of the disease filariasis and the virus of dengue fever. With this object, they were introduced into certain Pacific islands where they did not previously occur. This will be discussed in a later section as it has an important bearing on the present study which relates to the bionomics of *Megarhinus brevipalpis* (Theo.), based chiefly on insectary breeding of the species through several generations over a period of seven or eight months. *M. brevipalpis* has a wide distribution in the eastern part of the Ethiopian zoo-geographical region of Africa, from the Cape Province in the south to Kenya Colony in the north. The living specimens, from which the insectary colony was established in Johannesburg, were collected at Margate near Port Shepstone on the coast of southern Natal.

Observations in the Natural Habitat.

The habitat or habitats at Margate, where the species was first studied and collected, consisted of small patches of low evergreen forest, mostly isolated by being confined to narrow valleys or sheltered hillsides. Many of the specimens were collected in the Faerie Glen nature reserve, which was a small wooded valley near the centre of the Margate township area. The commonest trees of these habitats of the south coast of Natal are : *Albizzia gummifera, Protorhus longifolia, Sclerocarya caffra, Trichilia emetica,* and *Ficus* spp. There are also large monocotyledons such as the palms *Phoenix reclinata* and *Hyphaene crinita* ; also *Baphia racemosa, Dracaena hookeriana, Strelitzia nicolai* and others. The term " Palm Belt " has been applied to the vegetation.

Under the heading " Sub-Tropical Forest " Adamson (1938, p. 39) says the following : " This occurs on the low-lying strip along the Natal Coast, where rainfall and high temperature produce almost tropical conditions even in latitudes well outside the tropic itself. The type is confined to low-lying country nowhere over 1,000 feet (300 m.) and only occurs close to the coast ".

" The rainfall is 40–50 inches (102–127 cm.) though parts have only 35 inches (90 cm.). About 60–70 per cent. of the rain falls in summer, but there is no prolonged or distinct dry season. Temperatures are high and rather uniform, the mean winter temperature is not below 64°F. (17·7°C.), while in summer monthly means rise above 70°F. (21°C.). The annual range is 27°–33°F. (15°–18°C.). There is never frost ".

This same author gives the average rainfall, over 34 years, at Port Shepstone as 43·96 inches (111·65 cm.) and the mean minimum temperature (April–September) as 57·3°F. (14·0°C.).

Most of the evergreen trees of these small forests are of hard wood in which rot holes do not often form. Tree-holes, therefore, were not the chief breeding places of *M. brevipalpis* which is usual for this species in other parts of Africa. As noted above, a common plant associated with these wooded areas is *Strelitzia nicolai*, and this was very abundant in the habitats at Margate. This plant has a leaf and general form resembling that of the banana family, the leaves having very deep axils in which rain water easily collects. Certain *Aëdes* of the subgenus *Stegomyia* breed in the water of the *Strelitzia* axils, particularly *A. strelitziae* Muspratt (1950a). It was also in the leaf axils of this plant that most of the *Megarhinus* larvae were found. On one or two occasions they were collected from the small axils of *Dracaena hookeriana* where they preyed on larvae of *Aëdes* (*Stegomyia*) *simpsoni* (Theo.) and *A.* (*S.*) *deboeri* subsp. *demeilloni* Edw. Other breeding places were small tree-holes, and three larvae were once collected together from water in the stump of a *Strelitzia* plant that had been cut down.

As far as I know there are no previous records of *M. brevipalpis* breeding in plant leaf axils, apart from that of Haworth who collected larvae from water in the tops of coconut palms in East Africa (Edwards, 1923). Evidently this species of *Megarhinus* is quite adaptable as it is probably the scarcity of tree-holes which has made it take to axil breeding in southern Natal ; but there is some evidence of bionomical variation in the species from different parts of Africa which will be referred to below.

The *Megarhinus* larvae were withdrawn from the *Strelitzia* leaf axils by means of a strong rubber syringe bulb 2½ ins. in length by 1¾ ins. in diameter to which was attached a piece of glass tubing 8 ins. long, of ¼-in. diameter, and on the end of this a length of rubber tubing 22 ins. long with an internal diameter of ¼ in. The rubber tubing was inserted into the leaf axil and most of the water was withdrawn rapidly by suction from the rubber bulb. The water containing the mosquito larvae was then ejected into a white enamel bowl and the *Megarhinus* were removed with a wide aperture pipette. This same pipette was used to collect them from tree-holes and stumps. Others were collected by means of an artificial breeding place or trap. This consisted of a wide-mouth jar, 4 ins. across at the open end and 5¼ ins. in height, which was draped inside and outside with pieces of dead *Strelitzia* leaves. Water from a *Strelitzia* leaf axil, containing small *Stegomyia* larvae, was put into this so that there was an inch or two of water at the bottom of the jar. The draped jar was then placed at the foot of a tree where several *Megarhinus* males had been resting, and thereafter the trap was examined every three or four days.

The swarming or congregating of males has been noted by Howard & others (1912, p. 131). Some were caught and brought back alive to Johannesburg ; they were fed in transit on cane sugar solution. Sixteen larvae were transported to Johannesburg in corked specimen tubes measuring 1 in. across by 2 ins. in length, containing about ½ in. of water. All but one survived the shaking on the train journey of about 580 miles. It has since been found that larvae and pupae can be transported much more easily when out of water, if kept sufficiently damp. Small fourth-instar larvae have been sent by post as follows : A wad of cotton wool 1 in. in depth was put at the bottom of a specimen tube 1 × 3 ins. The larvae were stranded on the sides of the tube above the wad and two filter papers of 4¾ ins. diameter were crumpled up and put into the tube also above the wad. The cotton wool and filter papers were saturated with water. Two small grooves were cut in the cork of the tube to admit air.

Insectary Breeding and Behaviour of the Adults.

A larger number of males than females bred out from the original 16 larvae brought to Johannesburg ; and, after one or two accidents resulting from attempts to breed from the adults in cages which were too small, there were only two females left. This, however, afforded an opportunity of finding out the approximate number of eggs laid by a single female. Eggs were not laid when the adults were confined in a cage measuring 22 × 47 ins. and 25 ins. high. This was again confirmed with later generations. When released in the insectary, the original two females between them laid 173 eggs during three weeks ; the greatest number collected in a single 24-hour period being 32. They then stopped laying abruptly and did not live for more than three weeks after this, but as they had become damaged it is not known if they would normally have lived longer or recommenced laying. It would seem, therefore, that a female of this species can lay at least 85 eggs. This is more than double the number laid by females of *M. splendens* (Wied.) (during the period of eight days) when confined in comparatively small cages (Newkirk, 1947) ; but Paine (1934) considered that in the wild state oviposition continued for several weeks. Later observations with *M. brevipalpis* showed that eggs were laid six or seven days after emergence from the pupa, which agrees with the times given by Banks (1908) and Paine for *M. splendens.*

The insectary was a room measuring 9 ft. × 8 ft. 6 ins. and 9 ft. in height. It was below ground level and had a skylight 22½ ins. square covered with wire mosquito gauze. This skylight was open to the outside air in summer but closed with a frame containing glass during the coldest part of the winter. The temperature ranged from 25° to 30°C. (77°–86°F.), heat being derived partly from a heating installation in an adjoining room and partly from a thermostatically controlled electric radiator in the insectary. The humidity was usually high, particularly when the skylight was closed in winter, when 80 per cent. relative humidity was recorded.

Bamboo pots with an internal diameter of one to two inches were hung up near the skylight and in other parts of the insectary. The trap jar described in a previous section was also utilised to give the females an attractive place in which to oviposit. This time it was draped with dead leaves of the Canna lily. All these receptacles were filled or partly filled with water in which dead leaves were put to give them a rot-hole smell. Larvae of laboratory bred *Aëdes aegypti* (L.) were put in also, so that they would keep the surface free from any film which might have made the water unattractive to the gravid *Megarhinus*. Eggs were removed from the receptacles daily using a very fine water colour brush, a hand lens of × 5 and aided by an electric torch.

Various in-season fruits (grapes, peaches, apples, pineapple, citrus and tomatos) were hung up as food for the adults. Balls of cotton wool about the size of a table-tennis ball, were also suspended from the ceiling and frequently wetted with both cane sugar solution and honey mixed with water. The honey may not be absolutely essential as Newkirk (1947) obtained eggs from *M. splendens* using sucrose solution only, but the relationship of honey to the nectar of flowers suggests that its use is advisable in continuous insectary breeding.

Although rather variable in size, the adult males were comparable to those caught in the natural habitat and the females also appeared to be of normal size. One adult bred out was a gynandromorph (Muspratt, 1950b), having male appendages on one side of the head and those of the female on the other. The claws of the fore and middle legs were similarly bi-sexual. The wing on the side of the female appendages was shorter than the other, and the genitalia, although chiefly of the male, had features of both sexes.

Mating.

Mating was observed several times. The male and female usually clasped each other in the air afterwards falling to the ground where copulation was effected. On three occasions they were seen to be joined together by the genitalia whilst in flight with their heads in opposite directions, rather in the manner of copulating dragon flies. Nearly all the males habitually rested on the bamboo pots hanging in the centre of the insectary, or on wet rags which were hung above the pots as damp resting places for them. They made mating sallies from these when a female was on the wing, at the same time giving vent to their peculiar whining buzz. This whining note seemed only to be acquired some time after emergence. When the skylight was closed the females usually rested on the walls of the room. It is interesting to note that males of *Aëdes aegypti*, which were loose in the insectary, frequently tried to mob (or mate with ?) females of *Megarhinus* much to the annoyance of the latter which let out angry buzzing and occasionally they also molested the males.

Oviposition.

Oviposition was studied on a few occasions. When performed in flight this was as described by other writers. In one instance a female, with fore and hind legs raised, hovered over a bamboo pot of 1½ ins. internal diameter, and, with a swinging motion, giving rise to an undulating humming, dropped four eggs in quick succession, each time depressing the distal end of the abdomen. On a second occasion, a female laid eight eggs in two or three minutes on two different pots, with a short rest in between. On a third occasion, one descended into a glass jar (not draped with leaves), which was two inches across at the open end, and laid seven eggs in about a minute whilst at rest on the water at the bottom of the jar, four inches below the top. Eggs were sometimes found on the sides of the bamboo pots, above the water line, apparently having been laid there or shot against the sides.

The indications were that oviposition ceased when the temperature fell below 70°F. (about 21°C.). Before the insectary was closed for the winter, the females rested near the skylight exposed to the outside changes in temperature, and they did not lay when the outside shade temperature went below this figure. As the winter season progressed, oviposition became more and more intermittent until it practically ceased for quite long intervals in the middle of the winter. The temperature and humidity were kept constant during this period so it could not have been due to variation in this respect. Americano Freire and Faria (1947) encountered this same suspension of breeding activity with an insectary colony of *Anopheles darlingi* Root, and believed it to be brought about by the diminishing length of daylight during the winter months. An experiment was tried (by me) of keeping a light burning at night in the insectary for more than a week at this time, but it did not bring about an increase in oviposition. This might have had some effect if resorted to at the beginning of the winter (see also effect on larvae, p. 359).

During the period of minimum oviposition batches of eggs were laid containing some which were degenerate. These eggs were fertile but the developing larvae only reached a certain stage in the egg and then died, or died soon after leaving the egg. It is possible that this was caused by the reluctance of the females to oviposit and the eggs perhaps remained inside them until their vitality had become spent. In nature, the ova would probably not have matured at this time of the year on account of the lower temperature.

Immature Stages.

Growth and development.

A series of experiments were carried out to ascertain the average length of time taken in the immature stages of this species of *Megarhinus* and also the number of Culicine larvae usually killed or consumed. When given an abundant supply of the larvae of laboratory-bred *Aëdes aegypti*, the larval stage usually took two to three weeks. The shortest time was 11 days, when the fourth instar was reached in under six days after liberation from the egg. In one to three out of every ten larvae reared, the time in the fourth instar was unusually prolonged for some unaccountable reason which will be discussed below. Nevertheless, if these few with prolonged fourth instars, or more correctly, suspended pupation, are excluded, the average time of 50 larvae was 14·5 days. This is shorter than that estimated for *M. splendens* by Paine (1934) and Newkirk (1947).

Van Someren (1948) has noted that the larvae of *M. ruwenzori* van Someren, when near the pupal stage, usually killed a considerable number of their Culicine prey without eating them. This is the same with *M. brevipalpis* and it was therefore difficult to tell just how many *Aëdes* larvae were consumed for nutritional purposes. When the prey was killed but not eaten, it was a manifestation that the pre-pupal period of the fourth instar had been reached. It usually indicated that growth was completed and that the *Megarhinus* larva was about to pupate. In most cases this stage was not long, being one to three days. In one or two trials, 40–50 *Aëdes* were killed during two or three days of the pre-pupal period. Van Someren, however, gives the high rate of 10–20 larvae of *Aëdes simpsoni* killed per hour by *M. ruwenzori*. The habit, is, nonetheless, somewhat erratic as a few of the *brevipalpis* showed no inclination for the wholesale destruction of their prey.

In one example of an abnormally prolonged fourth-instar period, the pre-pupal condition was reached 14 days after hatching. The larva then began to kill the *aegypti* larvae without eating them but it did not pupate for 118 days (nearly four months) after this. During this time, it was given from five to ten fourth-instar *aegypti* every three or four days to prevent starvation, no attempt being made to achieve a record in the number of *Aëdes* larvae destroyed. Finally it had disposed of 80 *Aëdes* larvae during the development period and 162 during the extended pre-pupal period, many of the latter not being eaten. This larva may have had some constitutional defect, but the adult was quite normal. In some other cases of suspended pupation, the larvae practically ceased to kill their prey after a few days and remained like this for some days before killing began again. With this strange suspended pupation, if the larva did not pupate within three weeks from the first instar, the period in the fourth instar was usually prolonged for several weeks. These were all cases in which the larvae were not starved for even a short period, at least during development to the pre-pupal stage. Suspended pupation sometimes followed starvation tests, and a few of these larvae died, probably as a result of the starvation.

It should be stated that most of the prolonged larval stages were encountered when the temperature of the insectary was dropping below the minimum mentioned above due to a fault in the thermostat. The suspended pupation, therefore, may perhaps have something to do with winter survival in the larval stage. Further studies in the field would be necessary to determine this. It is also interesting to note that when the light was left on at night (see oviposition above) 18 out of 20 larvae which were in different periods of suspended pupation, pupated at about the same time. This may have been a coincidence, but perhaps in nature longer hours of daylight, as in summer, inhibit the prolonged larval life. This is strongly suggestive of a diapause analogous to that of larvae of *Anopheles plumbeus* Steph. (Wigglesworth 1947, p. 68). Against this is the fact that the breeding places of *Megarhinus* are mostly in deep shade.

Several small fourth-instar larvae have been kept out of water on damp filter paper in a closed petri dish for three and four weeks and afterwards most of them developed normally, except that with some, as stated above, suspended pupation followed full development. They appeared to keep in better condition out of water than when left in water and starved for the same period, as in the former state they remained immobile and did not expend any energy. After starvation, the appetite for *Aëdes* larvae was sometimes regained rather slowly ; nevertheless two fourth-instar *Megarhinus*, which had been starved (out of water) for three weeks, ate 132 and 119 *A. aegypti* larvae of the first and second instar in the four days following re-immersion. This indicates the destruction of newly hatched *Aëdes* larvae which might result if a *Megarhinus* survived a dry spell in the damp debris of a tree-hole (see discussion).

The table given below is for comparison with that of Paine (1934). The first six larvae in an experiment with eleven are taken to show the times in the four larval instars and the number of *Aëdes* larvae killed and mostly eaten except in the fourth instar. These Megarhines were reared in specimen tubes 1 in. in diameter by 2 ins. in length. They were each given up to 20 or 30 *Aëdes* larvae per day according to the number eaten in the previous 24 hours and these were approximately proportionate in size to the *Megarhinus*. Twenty or more fourth-instar *Aëdes* were sometimes consumed, in 24 hours, a day or two before the pre-pupal period. When this period was reached, indicated by the killing but not eating of the prey, the diet was restricted to five or ten larvae every two days to enable pupation to take place without killing an unnecessary number of *Aëdes* larvae. Nevertheless many of the *aegypti* larvae recorded in the fourth instar were killed without being eaten and were therefore not required for nutrition. This table, however, does not show the number of Culicine larvae which would possibly have been killed in nature : the total destroyed might be even larger. Three out of 11 *Megarhinus* larvae assumed long pre-pupal stages and did not pupate for several weeks ; these are not included.

In determining the larval instars the relative size of the head was usually the best indication, but the absence of the ventral brush in the first instar (see Breland, 1949) made it fairly easy to avoid confusion in the first two stages.

In the following table the numbers of *Aëdes aegypti* larvae killed by six *Megarhinus brevipalpis* are shown in brackets for each instar. Un-bracketed figures refer to the number of days.

Instar	1	2	3	4	5	6
1st	2 (14)	2 (15)	2 (15)	2 (16)	2 (13)	2 (16)
2nd	2 (23)	2 (27)	2 (29)	2 (29)	2 (23)	2 (25)
3rd	3 (35)	3 (43)	3 (47)	4 (47)	4 (47)	4 (43)
4th	6 (68)	6 (94)	7 (109)	8 (113)	7 (79)	7 (80)
Larval Total ...	13 (140)	13 (179)	14 (200)	16 (205)	15 (162)	15(164)
Pupal	5	6	5	5	5	5
Sex	♂	♀	♂	♂	♂	♂

Breland (1949) has reared *Megarhinus* larvae by feeding them, particularly in the later stages, on larval and adult fruit-flies (*Drosophila*). With *M. septentrionalis* D. & K.* it seemed that this variation in the diet was essential to make them pupate in a reasonable time. I have also used fruit-flies for the feeding of *M. brevipalpis* larvae which led to a rather interesting discovery. It was found that by employing a special technique the larvae would consume freshly killed and cut up fruit-fly maggots and one or two were reared from the first instar to the pupal stage on this diet alone. This showed that they were not absolutely dependent, in the laboratory, on being fed on living insects, and an interesting field of study was opened up to find out what insect tissues or other foodstuffs they could be reared. The fact that they will eat small pieces of raw meat and fat was mentioned by Jenkins and Carpenter (1946).

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*Classified as a subspecies of *M. rutilus* Coq. by Jenkins & Carpenter (1946).

My technique was to put newly hatched larvae into specimen tubes, 1 in. in diameter by 2 ins., with about ⅛ of an inch of water in the bottom. To the water was added the minced up insect tissues or other foodstuff and the tube was corked to prevent evaporation. The larvae moved about in this confined space browsing on the pieces of foodstuff. The water was changed daily and fresh food material put in, a pipette being used to move the larvae. Decaying food was found to form a film on the surface and to cause other complications (see below). As the larvae grew beyond the first two instars the amount of water was increased each day so that, when at rest on the surface, the head of the larva was nearly touching the bottom of the tube. In this way it could reach the food on the bottom and on the surface.

Three kinds of food materials were tried in experiments consisting of five to ten larvae in each, most of which were reared right through the larval life without any living insects, except when the experiment was abandoned owing to the prolongation of the larval stage due to unsatisfactory nutrition. The foods tested were (*a*) freshly killed and minced *Drosophila* maggots, (*b*) minced thorax and abdomen of freshly killed flies (mostly *Muscina* and *Sarcophaga* spp.) and (*c*) minced pork brawn. In experiment (*a*), out of five larvae, one only pupated in a reasonable time—this took 20 days ; in (*b*), all of six pupated in from 19 to 21 days ; in (*c*), one only out of ten pupated after a long time, and four others remained as small fourth-instar larvae for several weeks until fed on *Aëdes* larvae. The interest in the last experiment lies in demonstrating that one larva was able to develop through the larval life on a diet of mammalian tissue even though it was clearly an unsatisfactory food. The minced fly tissue appeared to be a most nourishing food, and the ability of the larvae to feed on this and probably on tissues of other insects is important as it might, in this way, be possible to keep a colony of them going at certain times of year when mosquito larvae were scarce. Unfortunately the technique is much more laborious than when using laboratory-bred *Aëdes aegypti* or other Culicine larvae, and there is reason to suspect that this food is not as good as would appear from the larval growth, as some of the adults showed signs of weakness.

In this connection it may be mentioned that one or two larvae were fed on fruit-fly maggots which had been dead for more than 24 hours. They ate these, but died before pupating, probably from bacterial infection of the gut. It seems that they are rather susceptible to these infections as shown in another experiment. In this, some were given *A. aegypti* larvae which had been reared on organisms derived from an infusion of cooked lentil meal, but several of them died when about to pupate. This mortality did not occur when they were fed on *A. aegypti* larvae reared by putting dead leaves in the rearing vessel.

Larval ecdysis, pupal metamorphosis and emergence from the pupa have all been observed. These transformations were essentially as in most other mosquitos. The shedding of the larval pelts was effected by the cuticle rupturing on the dorsum of the thorax first, so that the pelt could become detached from the head ; or in the case of pupation the internal part of the head receded. Before emergence, the pupal abdomen was raised parallel with the surface of the water as noted by Jenkins and Carpenter (1946).

The pupal stage took four to six days and never longer than six days irrespective of the length of the larval life. In the majority of a very large number of pupae recorded it was five days. This is, however, at variance with the findings of Garnham & others (1946) for this species in Kenya Colony. There, the pupal period was never less than six days (usually nine days) and sometimes prolonged to three weeks. Temperature may be a controlling factor.

Males and females emerged in about equal numbers : of 100 recorded 50 were females.

Immature Stages.

The egg and first-larval instar.

The eggs (fig. 1 *a*) were smaller than recorded by Banks (1908) for *Worcesteria grata* Banks* (? *M. splendens* (Wied.)) and of a more uniform size. They were about the same size as those of *Toxorhynchites immisericors* (Walker) (Green, 1905) which is considered to be a synonym of *M. splendens* and which agrees with the measurements given by Newkirk (1947) for that species. Five measured were between 0·56 and 0·58 of a millimetre in length and between 0·30 and 0·38 of a millimetre in width.

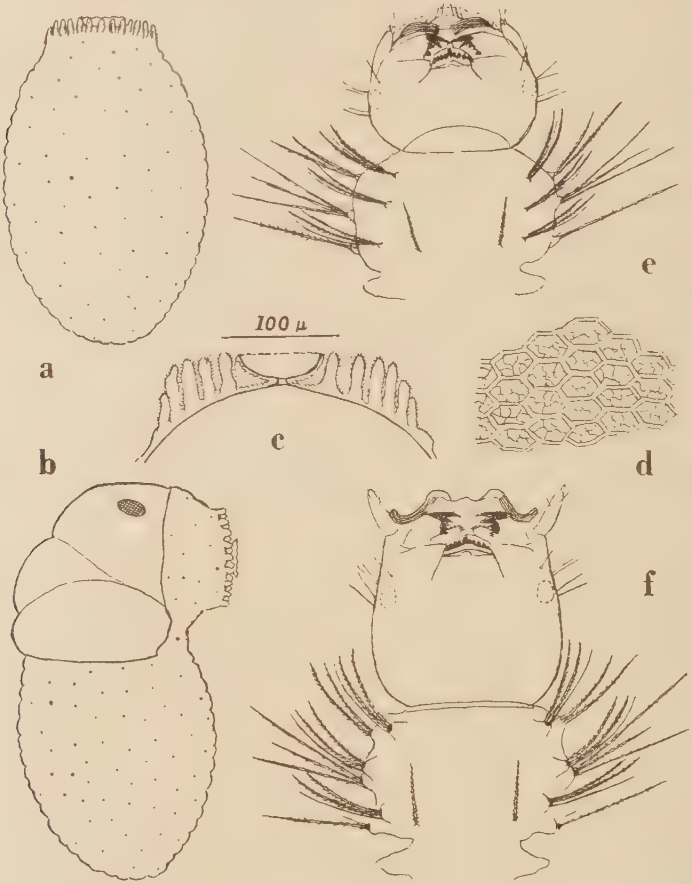

Fig. 1.—*Megarhinus brevipalpis.* (*a*) Outline of egg ; (*b*) diagram showing position of larva resting in egg-shell (drawn from living specimen) ; (*c*) cross section of upper part of egg showing projections of float crown and central cup ; (*d*) cells of exochorion of egg (same scale as *c*) ; (*e*) underside of head and thorax of newly liberated first-instar larva ; (*f*) first-instar larva two hours after liberation (viewed as in *e*).

They differed from those of the above species and from those of *M. septentrionalis* (Breland, 1949) in having numerous spiculate projections forming a flat crown at one end of the egg only (fig. 1 *c*) and were without the tubercles scattered over the entire surface. In the centre of the crown of projections was a cup-like structure (fig. 1 *c*). This did not appear to have an orifice connecting with the interior of the egg. It probably functions as a float by adhering to the surface film, as it was spiculate on the outside only. Measured on one egg it was $70 \cdot 4\,\mu$ across the top and $25 \cdot 6\,\mu$ in depth. The projections measured from $25\,\mu$ to $50\,\mu$ in depth and when viewed from above were most irregular in shape, some being in the form of ridges and others curved in a semi-circle.

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*This type series consisted of two different species (Barraud, 1934, p. 24).

The chorion consisted of two layers of which the endochorion was a plain yellow lining with faint striations and the exochorion, which was unpigmented, was made up, apart from the float crown and cup, of cells. Many of these cells were roughly hexagonal (fig. 1 *d*) giving the outside of the egg the appearance of a honey-comb pattern ; their sides were slightly raised.

When first laid the eggs floated high in the water but later became submerged except for the float crown. At first they were creamy white, but afterwards turned yellowish and finally greyish from the larval hairs showing through (noted by Newkirk, 1947). When dropped into the water (if more than 24 hours old) they sank below the surface but were immediately brought back again by the buoyancy of the float crown. Larvae were ready to hatch out within 40–50 hours after the eggs were laid, where oviposition had been observed, and the larvae wriggled away rapidly from the egg-shell after hatching when in water.

It is known that *Megarhinus* eggs, unlike those of many *Aëdes*, will not survive desiccation even after the larva has developed. The following experiment was, however, made to see if the larvae in the egg-shell would survive when in a damp atmosphere and on a damp surface. Some eggs were removed from the surface of the water on which they had been laid, before they were 24 hours old, being lifted with a fine water-colour brush. They were then placed on a damp, but not wet, surface in a petri dish which was kept closed so as to maintain a saturated atmosphere. Like those left in water the eggs hatched with the chorion splitting open at about one-third below the float crown. The larvae, however, did not extricate themselves from the shell but remained in the position shown in fig. 1 *b*. The head was bent over in the upper part of the shell which remained attached, at right angles, to the lower part containing the abdominal segments. The neck part, between the dorsum of the prothorax and the head, became very distended and it seemed that this might serve some respiratory purpose, as the newly hatched larvae had two large air sacs above the heart and connected with the tracheal system (illustrated by Green, 1905). The larvae moved frequently while in this position, but made no attempt to leave the egg-shell unless placed in water when they immediately wriggled free. In the course of a number of experiments it was found that larvae would survive like this, in a saturated atmosphere, for four or five days, a small percentage surviving even up to six days. This was about the same time that first-instar larvae survived in tap water if given no food after hatching from the egg.

The success of the above experiment depends on the egg being placed on a surface which is only slightly damp ; if it is too wet the larvae will leave the egg-shell. The technique was to cut a filter paper, 4¾ ins. in diameter, in two and to fold each half so that the pieces could be placed in a petri dish of 4 ins. diameter. These pieces of filter paper were wetted. The eggs were then transferred to a small piece of filter paper which was made damp but not too wet, and this was placed on three match sticks on the wet pieces of filter paper in the petri dish. In this way, the eggs on the damp surface were raised above the wet filter paper, and, when the lid was on, they were thus suspended in a saturated atmosphere. If the lower pieces of filter paper showed a tendency to become dry after a day or two, a few drops of water maintained their humidity.

When first-instar larvae were liberated in water, either immediately after rupture of the egg-shell or after a resting period in a damp atmosphere, they were just over 1 millimetre in length and the head was quite small and almost spherical (fig. 1 *e*). The mouth brushes and mandibles were folded in and the thoracic bristles were folded round the thorax. Soon after immersion the thoracic bristles assumed the position shown in the figure. The head then began to enlarge and the mouth brushes were pushed forward on two projections from a vertical to a horizontal position, the detached ends at the same time being turned outward. In fig. 1 *e* the mouth brushes are seen almost end on with the curved free ends turned inwards underneath the head. The antennae also are bent downwards (upwards in the figure). Afterwards the mouth brushes came up into the position shown in fig. 1 *f* with the mandibles also coming to a slightly more forward position and the sub-basal promontories of the thoracic bristles becoming much enlarged. The transformation was completed within about two hours, when the larval head and thorax ultimately resembled fig. 1 *f*. The larvae were then ready to catch their prey which they did by snapping at newly hatched larvae of *Aëdes aegypti* until one was caught. In an experiment with six larvae which were put into jars of water containing dead leaves, but no Culicine food, they survived from a few days to over four weeks, and four reached the third instar probably by feeding on the larger micro-organisms. One which was given Culicine larvae after five weeks completed its development.

The first observations above, however, conflict with the finding of Banks (1908), who states that the larva of *Worcesteria grata* (probably *M. splendens*) remains unchanged except in colour, and he adds : " The distinctive appearance of the head and lateral bristles and spines remains the same throughout the larval existence." Green (1905) also apparently did not observe this transformation.

Cannibalism and reversed Predacity.

There appears to be some difference of opinion as to whether *Megarhinus* larvae readily devour one another or not. Edwards (1932, p. 59) says : " The larvae are very voracious and will readily attack other members of their own species hence it is unusual to find more than one *Megarhinus* larva in each breeding place." Morgan and Cotton (1908), however, kept several larvae of *M. septentrionalis* together in the same vessel during prolonged starvation without cannibalism taking place and this has been repeated by Jenkins and Carpenter (1946). Barraud (1934, p. 13), Paine (1934) and Newkirk (1947) regarded *M. splendens* as having little or no powers of discrimination and this species apparently resorts to cannibalism particularly in the absence of Culicine larvae. Hopkins (1936) referring to *M. brevipalpis* stated that : " I have found *Megarhinus* very reluctant to eat members of their own species, though they will do so if no other food is available." The fact of the matter may be that the cannibalistic tendency probably varies with different species and also depends on the size or relative size of the larvae. My experiments indicate that for *M. brevipalpis*, Hopkins' statement is generally correct, except that the size of the larvae is an important factor as the following tests show :—

1. Three third-instar larvae which were fairly hungry but not starved were put into a dish measuring $4\frac{3}{4} \times 6\frac{3}{4}$ ins. Attracted by each other's presence they stalked one another with reactions similar to those described by Breland (1949). When two came in contact they seemed fully aware that they belonged to the same tribe, and after touching one another they moved quickly away. When larvae of *A. aegypti* were afterwards put into the dish they were immediately seized.

2. Six first-instar larvae were put into a jar measuring $3\frac{1}{2}$ ins. across with $3\frac{1}{2}$ ins. of water. They were supplied with a large number of first-instar *aegypti* larvae and, to enable these to grow, dead leaves were put into the water. Two of the *Megarhinus* disappeared by the time the remaining four had reached the fourth instar, when the *aegypti* larvae were also finished. The four fourth-instar larvae were then left together for several weeks, but they did not eat one another.

3. A fourth-instar larva was put into a jar as in experiment 2, except that six others of the first instar were put in with it in addition to the first-instar *aegypti* larvae. Two only of the small Megarhines survived to reach the fourth instar, when

all the *aegypti* had also been eaten. One of the smaller fourth-instar Megarhines disappeared 11 days later, but the remaining two were left together for over a month without any food. Probably the original fourth-instar *Megarhinus* ate most of the *aegypti* and smaller Megarhines, but apparently they were insufficient food to enable it to pupate.

4. Seven first- and second-instar larvae were put into the same jar as in the other two experiments but without any *Aëdes* larvae. Four remained a day later and two only four days after this ; the last two having passed through to the fourth instar by cannibalism only. These were kept together and one of them pupated 20 days later without any more food. The other was then given *Aëdes* larvae which it began to eat immediately.

It will be seen from the above that in the early larval instars cannibalism is readily resorted to, also that fourth-instar larvae have no powers of discriminating between their Culicine prey and small larvae of their own species. The fact that Megarhine larvae in the first two instars can reach the fourth instar and pupate by devouring each other has considerations with regard to biological control which will be discussed later. In this connection, a more serious matter is the possible destruction of newly hatched Megarhines by fourth-instar Culicine larvae. In the case of cannibalism by Megarhine larvae at least one would survive in the breeding place ; but Macgregor (1915) observed that large larvae of *Aëdes aegypti* would consume small members of their own species, and it seems that newly hatched Megarhines may occasionally fall victims to this predacity. In 40 tests in which single newly hatched Megarhines were put into jars with *Aëdes* larvae in all stages of growth, five disappeared probably on account of this reversed predacity. Nevertheless in nature this might not be of much account as several eggs would, in most cases, be laid in each breeding place. It is also not quite certain if this was the cause as there was sometimes a small percentage mortality amongst apparently healthy larvae.

Discussion.

The chief practical interest in this remarkable genus of mosquitos lies in the attempts that have been made to use them in the biological control of disease carrying Culicines. In certain Pacific islands, they have been specially introduced with great care for this purpose and their introduction has been recommended for other Pacific islands where mosquito-borne disease is a menace to public health.

As a result of their researches in Polynesia and Melanesia, Buxton and Hopkins (1927, p. 121) recommended the introduction of *Megarhinus* as a means of reducing the carriers of filaria, particularly in the Islands of Western Samoa. There, *Aëdes pseudoscutellaris* (Theo.) was the principal vector and a prolific breeder in the forest areas where a *Megarhinus* could be expected to establish itself.

The first attempt at such an introduction was made by Pemberton in Hawaii (Swezey, 1930). *M. inornatus* Walker was brought from New Britain with the idea of reducing the breeding of the dengue vector *Aëdes albopictus* (Skuse). This Megarhine was bred by providing the adults with special breeding places (barrels or tubs) in the wooded areas where it had been first released. In this way immature stages were collected for further introduction into other areas. This species did not, however, thrive under natural conditions and finally died out (Swezey, 1931 ; Williams, 1931, p. 279).

The same method was adopted by Paine (1934) in Fiji. *M. inornatus* was brought there also from New Britain, and later *M. splendens* was imported from Java in the Dutch East Indies. Paine was unsuccessful in breeding the latter species in an insectary but established several colonies by Pemberton's method. This species became established, but not very abundant, and it was even carried over a considerable stretch of water to an island where it had not been introduced (Lever, 1938, 1941).

The most optimistic estimates of its use in reducing the filaria-carrier, *A. pseudoscutellaris* were, however, extremely low. Newkirk (1947), investigating the efficacy of *M. splendens* in New Guinea and the Philippine Islands, considered it of little value, chiefly on account of its slow rate of increase and self destruction by cannibalism.

It is therefore clear that unassisted biological control with the species of *Megarhinus* used hitherto in the places where they have been introduced, or where they occur naturally, is of small value. The writer would nevertheless like to suggest that the idea of using these insects for this purpose should not be abandoned. Points for reconsideration are : (*a*) Could their efficiency be increased by breeding them in an insectary and releasing them in large numbers at suitable times of year as a continuous measure, irrespective of their natural increase or dispersal ? (*b*) Because certain species have proved failures or of only small value in certain Pacific islands are there not others from other parts of the world which could achieve the desired object ? Their effect might be more marked on some of the smaller islands or in circumscribed areas, particularly when used as part of a general control programme.

One of the advantages of insectary breeding over the field method evolved by Pemberton and further developed by Paine is that, theoretically at any rate, by isolating each larva (starting from the egg) it should be possible to breed up very large numbers in a comparatively short time for mass release. A simple calculation shows the possibilities. If we regard the life-cycle of *brevipalpis*, from adult to adult, as being usually three or four weeks, and we start with ten females, which we will assume lay 85 eggs each, after the first six weeks or so we should have about 850 adults. Of these about 425 would be females, which, after another three or four weeks should be capable of producing 36,125 larvae. By restricting the diet of those larvae which hatched first, it should be possible to arrange it so that large numbers of adults would emerge, after a further three or four weeks, within a few days of each other. Thus rapid mating would be assured and the females would then seek out tree-holes, plant leaf axils, etc., in which to oviposit.

It is obvious, of course, that it would not work out quite like this in practice, even if the insectary (or insectaries) were large enough, and sufficient Culicine larvae were available. There would always be a certain mortality, sterility and other drawbacks, as well as a certain percentage of larvae with a prolonged larval life ; but the latter could perhaps be prevented when the reason for it is better understood. It must also be admitted that it is not yet proved that *brevipalpis* or other *Megarhinus* species will survive prolonged insectary breeding. Until our knowledge of this has improved it would probably be advisable to continue with the field method of breeding or use a combination of the two methods. Using Pemberton's method, as described by Paine (1934), eggs could be collected and isolated perhaps in jars of about 3 ins. diameter, and as the Megarhine larvae hatched they could be given newly hatched Culicine larvae. Dead leaves could be put into the jars so as to enable the Culicine larvae to keep pace in growth with the Megarhines as would occur in nature ; or the diet could be restricted as desired.

It would seem that the strain of *brevipalpis* from southern Natal has certain advantages over *splendens*. The most important is the usually shorter larval period. Also the survival of the first-instar larva in the egg when on a damp surface, and the survival of larger larvae out of water ; the latter would probably live for at least a few days in the dead leaves and damp debris of even quite small tree-holes and other breeding places when these had " dried out ", perhaps sufficiently long to see them refilled by more rain.

It is difficult to say if an occasional prolonged larval life may not sometimes be an advantage when considering biological control by Megarhines. If the larva was in a fairly large rot-hole of a tree, great destruction of Culicine larvae might result. Garnham & others (1946) give a record of 195 fourth-instar larvae of *A. aegypti* consumed by a single *M. brevipalpis* in 12 days before pupation. Where small tree holes or leaf axils are concerned, a long larval period would probably be a disadvantage, as the breeding place might dry out completely before the larva was able to pupate. Even so, if it was a fourth-instar larva it might survive as noted above, and when in a leaf axil perhaps it would obtain sufficient moisture from the plant to remain alive for longish periods in these circumstances. These suggestions are, however, only surmises as they have not yet been investigated.

The survival of the larvae out of water is also important in considering the transportation of larvae from one place to another. With modern airmail communications it would seem that they could be sent to quite distant destinations, in view of their survival for at least two or three weeks in a damp atmosphere, provided they were not subjected to big changes in temperature. With a view to transportation of *Megarhinus* from one country to another Wigglesworth (1929) cites an instance of a larva surviving for five months on a restricted diet and there are other records of long survival during starvation (Morgan & Cotton, 1908 ; Schwetz, 1930). Paine, however, stated that after prolonged starvation of seven weeks larvae of *splendens* began feeding again but died after four weeks. Some apparently were unable to withstand starvation of three days, but in one instance a larva matured normally after three weeks' starvation. It would appear from this and from my experiments that three or four weeks is about the limit of complete starvation for normal recovery.

Cannibalism may certainly be a disadvantage if the larvae were in a large tree-hole or large artificial container and attacked one another on coming in contact. In a small breeding place, however, apart from the wastage in the number of eggs laid, it might be advantageous for all the larvae to be killed off except one, so that the remaining one could pupate in a short time. Mr. J. J. Steyn (unpublished report, 1950) found 15 *brevipalpis* larvae in a discarded bucket near Durban (Natal).

Conclusions.

Newkirk (1947) pointed out the very big difference in the estimated reduction of harmful *Aëdes* by *M. splendens* (Paine, 1934) and the spectacular results achieved by aircraft-sprayed DDT in the Philippine Islands. One must not forget, however, that in these days of post-war economic stringency in many countries, there are few places where complete eradication programmes can be undertaken. Davis (1949) has shown that the older methods used in combination have produced effective control of *pseudoscutellaris* in the Cook Islands. Tredre (1950) in reviewing this latter paper says : " this paper is a useful reminder in these days of powerful and expensive insecticides that the older simple hygiene measures can be most effective, if properly organized, and may clear the path for a rapid eradication campaign ".

The suggestion is therefore made here that biological control by laboratory-bred Megarhines should be tried as an auxiliary method. Native personnel could probably be trained to do the insectary work. *M. brevipalpis* would appear to be a suitable species, particularly those strains which breed in small tree-holes and leaf axils ; but there may be other species which would be even better. Unfortunately they would be of no avail against those vectors which breed in rock pools and crab holes unless, by insectary breeding or the release of pupae in these breeding places, the adults could be " educated " to return to them to oviposit ; but this is rather doubtful.

The writer has been in communication with Mr. C. E. Pemberton in Hawaii concerning the attempted introduction there of *M. inornatus*, and it has been decided to try further experiments with *brevipalpis* by sending airmail consignments of living larvae from Johannesburg to Honolulu. The introduction will be carried out under the direction of Dr. Stephen M. K. Hu and Dr. David D. Bonnet of the Territory of Hawaii Department of Health.

So far a trial consignment of 22 larvae has been sent, packed as previously described. Most of these arrived alive but there were some losses from cannibalism owing to close packing. A second consignment of over 100 larvae was dispatched of which 66 survived and it has since been learned that the colony is breeding well in the insectary near Honolulu.

Summary.

Living specimens of *Megarhinus brevipalpis* were transported from southern Natal to Johannesburg to establish an insectary-bred colony. The natural habitat of these predatory mosquitos consisted of small isolated patches of sub-tropical forest, in which the rainfall is 40–50 ins. (102–127 cm.) with a mean winter temperature of 64°F. (17·7°C.) and an annual range of 27°–33°F. (15°–18°C.). The breeding places were leaf axils of *Strelitzia nicolai* (a plant resembling a wild banana), small rot holes in trees and larger ones in *Strelitzia* stumps. The larvae were collected from leaf axils with an apparatus consisting of a rubber bulb to which were attached lengths of glass and rubber tubing.

The insectary was a room 9 ft. × 8 ft. 6 ins. and 9 ft. high which was kept at tropical heat and humidity. Mating of the adults was observed, copulation being effected while at rest or in flight. Oviposition was usually accomplished in flight but also while at rest on the surface of the water. In the summer time two females, which were tested, laid about 85 eggs each during the month following emergence from the pupa, six or seven days elapsing after emergence before the first oviposition. In the middle of the winter, oviposition (with later generations) became very irregular in spite of the temperature and humidity remaining constant. The adults, which were comparable to those of the natural habitat, were fed on sugar solution, honey and fruit juice. One bred out as a gynandromorph.

When given an abundant supply of larvae of laboratory bred *Aëdes aegypti*, the life-cycle of *M. brevipalpis* was normally : egg (incubation), less than two days ; larva, 11–20 days (average 14·5 days) ; pupa, five days. This does not include a small number of exceptional cases in which the life as a fully grown larva was abnormally prolonged (in one case nearly four months) for reasons which are not absolutely clear. The larvae killed from 100 to 200 or more *Aëdes* larvae during the normal larval life, but many of these were not eaten when the *brevipalpis* were in the late fourth instar. By a special technique they were also induced to eat dead tissues including minced pork brawn, minced maggots and minced flies. Except for the latter these were not satisfactory foods although there was slow development.

Fourth-instar larvae were kept out of water for three to four weeks (without food), in a damp atmosphere, and afterwards when fed most of them developed normally, but pupation was sometimes suspended for a considerable time. They have been sent by post (out of water) in tubes with damp cotton wool and filter paper.

The egg differed from that of other *Megarhinus* species in having a crown of projections at one end with a cup-like structure in the centre. The exochorion had roughly hexagonal cells but without numerous tubercles as in other species.

First-instar larvae remained in the egg-shell after hatching when the eggs were out of water but on a damp surface and in a saturated atmosphere. They survived like this for up to six days or about the same time as the larvae survived in tap water if there was no food. When liberated in water the head of the first-instar larva was comparatively small with the mouth parts folded in. Within two hours of liberation in water the head enlarged considerably and the mouth parts came into position ; the larva was then ready to catch its Culicine prey. When in water containing dead leaves, these larvae survived from a few days to over four weeks and some grew to the third instar without any Culicine food.

Cannibalism was investigated. Fourth-instar larvae did not attack each other readily ; they devoured smaller larvae of their own species and small to medium size

larvae resorted to cannibalism, particularly in the absence of Culicine prey. There was evidence that fourth-instar *Aëdes aegypti* occasionally ate first-instar *Megarhinus*.

The discussion traces attempts which have been made in certain Pacific islands, notably Hawaii and Fiji, to use Megarhines for biological control of disease-carrying mosquitos. *M. brevipalpis* has a shorter life-cycle than the species introduced into these islands and the conclusion reached is that laboratory breeding, to enable large numbers to be released in certain areas, would be a suitable adjunct to a programme of general control, in this part of the world. Airmail consignments of larvae are being sent to Hawaii with the object of starting a laboratory colony there.

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SEASONAL VARIATION IN THE INCIDENCE OF *TRYPANOSOMA VIVAX* IN *GLOSSINA PALPALIS* (R.-D.).

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In a recent paper (Squire, 1950), the results are recorded of an investigation in which some 5,000 examples of *Glossina palpalis* (R.-D.) were classified into three age-groups according to wing colour and fraying over a period of a year. Thus any shifting of the average age of the fly population can be measured by means of the percentage of flies in the different age-groups. Moreover, it was pointed out that unmated females can be distinguished by the absence of mating scars. Practically all females mate within a few days of emergence, judging by the fact that all unmated females are extremely teneral. Hence the percentage of unmated females (expressed in this case as a percentage of the females in Age-group 1) furnishes a good index of the rate of emergence at any particular period.

Of the flies thus classified, 3,226 were dissected in order to determine the incidence of infection in the proboscis, in the mid-gut and in the salivary glands. In this note, discussion is confined to infection by the *Trypanosoma vivax* type, *i.e.*, the type found only in the proboscis. The work was carried out at Njala, situated on the Taia river, approximately 8°5′ N. and 12°5′ W. Previous work in other parts of Sierra Leone is summarised below.

TABLE I.

Authors and date of work	No. of flies dissected	Per cent. infected with *T. vivax*
Yorke & Blacklock (1915) Dec. 1914–Feb. 1915	400	3·75
Gordon (1928) July–Aug. 1928 ...	209	16·17
Gordon & Davey (1930) Sept. 1929...	986	8·3

It will be observed that the highest incidence occurs in the rains, and the lowest in the dry season. In Nigeria Johnson and Lloyd and their co-workers (1923, 1924) found that the incidence of *T. vivax* in *Glossina morsitans* Westw. and *G. tachinoides* Westw. increased as the rains progressed. This they attributed to the increasing average age of the fly population due to the gradual decline in the rate of emergence following the onset of the rains. But as they had no exact means of comparing the average age at different periods, or of measuring the rate of emergence, their conclusions remained open to doubt.

The present investigation covered the period September 1948 to August 1949. All the flies were age-grouped before dissection, and the rate of infection recorded separately for the different age-groups. Thus the age factor in the incidence of infection was eliminated. The results are given below in Table II and fig. 1.

It will be seen that, excepting September and October, the months of heaviest infection, the incidence in Age-group 1 is zero. Therefore, for practical reasons, it is expedient to omit this age-group in dissecting. Age-group 3 has consistently the highest incidence and would by itself furnish a reliable index of infection but for its paucity at times. Age-group 2 affords the best index. It will be observed that the incidence in Age-group 2 and Age-group 3 is up in the rains and down in the dry

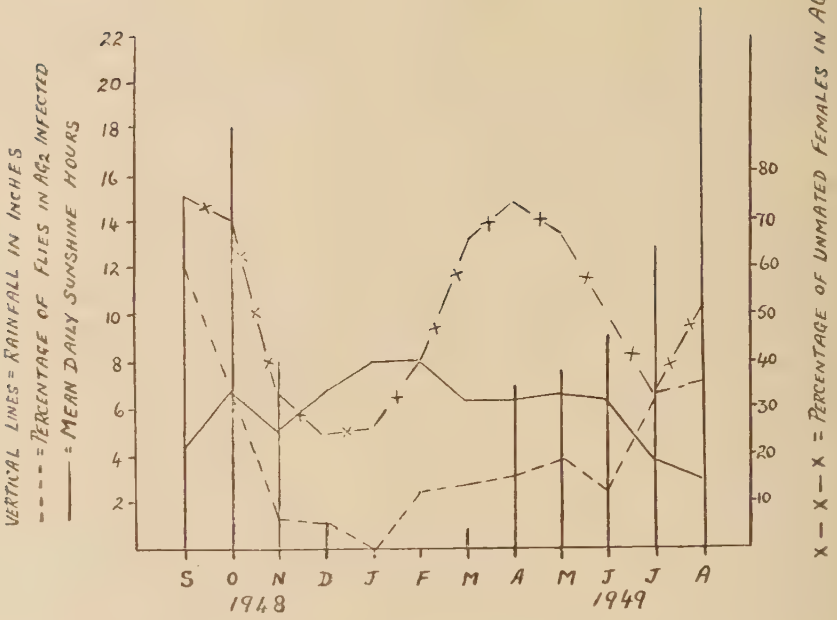

Fig. 1.

season. Here there is no question of "dilution" at certain periods by newly emerged flies which have not had a chance to become infected because they have been excluded in Age-group 1. It does not look, therefore, as if the explanation of the seasonal fluctuation in infection is to be found in the average age of the fly population. Considering the percentage of unmated females in conjunction with the incidence of infection, it is seen that during the months of August, September and October when the incidence of infection was highest, even if all the flies of the different age-groups are lumped together, there was actually a peak in the rate of emergence which should tend to lower the average age, and hence the incidence of infection. But in spite of this masking effect the incidence is still highest. This again shows that the high incidence of infection in the rains cannot be explained merely as due to the increased average age of the fly population. It evidently has significant external causes which are unknown.

The purpose of this paper is to draw attention to the practical importance of the phenomenon and to show that the explanation is by no means as simple as has hitherto been supposed. From fig. 1 it will be seen that there is evidently a strong correlation between seasonal climatic factors and the incidence of infection, positive in the case of the rainfall and negative in the case of the amount of sunshine. The latter, it will be observed, is a remarkably close correlation. The significance of this is by no means clear, but it must be remembered that *T. vivax*, lodging as it does in the labral cavity, is possibly subject to atmospheric conditions which may prove unfavourable at times, especially by reason of the normal infrequency of blood meals which, by providing a fluid medium, would guard against desiccation for a short time while plasma remained in the proboscis. No doubt the influence of

TABLE II.

Year	Per cent. infected						Number dissected. Number infected in brackets			
	A.G.1	A.G.2	A.G.3	M	F	T	A.G.1	A.G.2	A.G.3	T
1948										
Sept. ...	2·6	11·9	14·6	10·3	8·5	9·1	116 (3)	176 (21)	48 (7)	340 (31)
Oct. ...	3·7	7·1	20·0	7·8	9·8	8·4	54 (2)	70 (5)	30 (6)	154 (13)
Nov. ...	–	1·3	5·1	1·8	1·9	1·8	85 (0)	158 (2)	78 (4)	321 (6)
Dec. ...	–	1·1	5·5	1·6	1·2	1·5	144 (0)	175 (2)	73 (4)	392 (6)
1949										
Jan. ...	–	–	1·8	–	2·5	0·5	75 (0)	90 (0)	55 (1)	220 (1)
Feb. ...	–	2·6	6·3	1·4	5·6	2·2	34 (0)	39 (1)	16 (1)	89 (2)
Mar. ...	–	2·8	4·3	2·8	1·3	2·2	116 (0)	176 (5)	69 (3)	361 (8)
Apr. ...	–	3·3	10·0	5·5	5·9	5·6	8 (0)	60 (2)	40 (4)	108 (6)
May ...	–	3·8	5·0	4·1	–	3·5	23 (0)	52 (2)	40 (2)	115 (4)
June ...	–	2·5	15·9	3·8	1·7	3·7	105 (0)	122 (3)	44 (7)	271 (10)
July ...	–	6·8	14·5	4·8	9·1	6·5	104 (0)	205 (14)	76 (11)	385 (25)
Aug. ...	–	7·0	12·3	4·7	7·1	5·5	149 (0)	256 (18)	65 (8)	470 (26)
							1,013 (5)	1,579 (75)	634 (58)	3,226 (138)
	Per cent.						0·5	4·7	9·1	4·3

Males ... ... Total 2,313, no. infected 85, per cent. infected 3·7
Females ... ... „ 913, „ „ 53, „ „ 5·8

Total ... ... 3,226 138 4·3

atmospheric conditions is mitigated by the extreme proximal siting of many of the colonies in the proboscis and also by the general high humidity of the fly's haunts.

Attention should be drawn, however, to the slight drop in the incidence of infection in Age-group 2 in the months of heaviest rainfall, *i.e.*, October (18·07 ins.) and August (22·58 ins.). The highest incidence was recorded in September (14·94 ins.).

It will be seen that there is a difference, but one of doubtful significance, between the incidence of infection in the males and the females.

It may be of interest to compare the percentages of infection obtained during this investigation with those given by Lloyd and Johnson (1924) in Nigeria. These are summarised in Table III.

TABLE III.

	Lloyd and Johnson Jan.–Dec. 1923		Present investigation
	G. morsitans	*G. tachinoides*	*G. palpalis*
Minimum ...	8·0	2·4	0·5 (Jan. 1949)
Maximum...	23·0	7·4	9·1 (Sept. 1948)
Mean ...	15·1	4·2	4·3 (Sept. 1948–Aug. 1949)

In a previous paper, Johnson and Lloyd (1923) state that the incidence of infection in *G. tachinoides* is approximately twice that in *G. palpalis*. But this conclusion was based on rather limited data. It will be seen that the incidence in *G. palpalis* in Sierra Leone is comparable with that in *G. tachinoides* in Nigeria, but that there is a wider fluctuation.

In view of the well-known importance of food in connection with the incidence of infection, it is necessary to give a brief mention of the situation in the environs of Njala where the flies were taken. The latter are confined to the narrow strip of forest along the Taia. The area is densely populated, with the result that big game is scarce. The main food supply is judged to be man and his domestic animals, monkeys, and possibly reptiles and rodents. None of these varies seasonally, so that they do not provide an explanation for the fluctuating rate of infection.

The failure to recognize seasonal differences in the incidence of infection has led to some practical misconceptions. Thus Gordon and Davey (1930) whose observations were made in July and August, 1928, concluded that there had been an important rise in the general status of the parasite since Yorke and Blacklock (1915) had made their observations in December, 1914 to February, 1915. Other observers, basing their conclusions on dry season observations, have pronounced Sierra Leone eminently suitable for cattle raising—on this score, an unduly optimistic opinion.

The method of dissection for the proboscis employed was that described by Lloyd and Johnson (1924), but for the gut and salivary glands a new technique was evolved. The head having already been severed for the purpose of the proboscis dissection, the mid-gut and salivary glands were easily drawn out from the pleural region between the second and third abdominal segments by means of a minute hooked needle, thus avoiding further tiresome dissection.

Summary.

Over 3,000 examples of *G. palpalis* were age-grouped and dissected for *T. vivax* over a period of a year. The incidence of infection throughout was highest in Age-group 3 and lowest in Age-group 1. There was a marked seasonal fluctuation, the incidence being highest during the months of heavy rainfall, *i.e.*, August to October, and lowest in the driest.

It has been suggested that the rise in the rate of infection during the rains is merely due to the increasing average age of the fly population, but by the method of age grouping it is shown that this is not the case.

The degree of infection in *G. palpalis* in Sierra Leone is comparable with that of *G. tachinoides* in Nigeria.

It is not thought that the seasonal variation in the incidence of infection can be ascribed to changes in the type and abundance of food. The true cause of the fluctuating incidence of infection like the source of infection is unknown.

The failure to recognize seasonal differences in the rate of infection has led to some important practical misconceptions.

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THE EFFECT OF PARATHION DUSTS ON *EURYGASTER INTEGRICEPS* PUT. (HEM.).

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Like many other countries in the Middle East, Syria suffers greatly from the Wheat Shield Bug, *Eurygaster integriceps* Put., locally named "Souni". This Pentatomid is capable of causing tremendous losses to the wheat grower, and the Government spends large sums of money annually in rewards for collecting the overwintering adults, the egg masses, and the spring nymphs, with the object of minimising the damage caused.

Many control methods have been tried. The use of nicotine, petroleum emulsions, sulphuric acid, and calcium cyanide has not produced satisfactory results (Achard, 1927) ; in Russia, good results have been claimed for calcium arsenite dusts, sodium arsenite solution, and sodium nitrite solution (Starostin, 1944) ; running poultry in infested areas has also been mentioned (Arnoldi, 1943). In Syria, the Russian methods cannot be put into practice, because the areas attacked are remote from any water sources for spraying. Mechanical methods have therefore been resorted to, as in Persia (Achard, 1927), the Crimea, Irak, and Turkey. These consist essentially of sweeping the wheat ears with sweep nets 30–40 cm. in diameter, the insects so collected being burnt. The leaves of the weed, *Leontice leontopetalum*, on which the insect lays its eggs, are also collected and burned before the eggs hatch.

After the recent war, benzene hexachloride dust was tried at different concentrations in the field and the laboratory but proved unsatisfactory and mechanical methods had to be continued.

Laboratory Tests.

Early in 1950, the Ministry of Agriculture received small quantities of parathion dusts for experimental purposes. Laboratory tests were performed by dusting glass cylinders with a 0·8 per cent. pure parathion dust at the rate of 0·1 mg./sq. cm. Newly hatched nymphs of *Eurygaster* were introduced into the dusted tubes and kept for three hours in contact with the dust before removal to clean tubes. The results after 24 hours are shown in Table I.

TABLE I.

Dusted (0·8 per cent. parathion)				Control			
Lot	Dead	Moribund	Live	Lot	Dead	Moribund	Live
A	9	12	0	E... ...	3	2	22
B	9	26	0	F... ...	7	0	9
C	4	18	0	G... ...	4	4	15
D	7	11	0				
Total ...	29	67	0	Total ...	14	6	46

Dead.—When no movement of any appendage can be seen under 16× magnification.

Moribund.—When very little movement of the appendages can be observed under 16× magnification.

Live.—When the insect can walk freely and normally after release from the tubes.

It will be seen that 24 hours after dusting, there were no survivors from the dusted tubes, whilst in the control tests nearly 70 per cent. survived. It should be added that 48 hours after dusting the 67 moribund dusted individuals were all dead whilst in the control 18 were dead, 12 moribund and 36 alive.

In a second experiment two cages each 30×30×30 cm. were dusted at a rate of approximately 10 kg./hectare. The dust was applied by means of a knapsack duster provided with an accurate regulating device. This device was set in such a way as to give the minimum amount of dust at a uniform rate, capable of covering a measured area with a weighed quantity of the parathion dust. The one cage contained 1,300 adults and the other 150. Similar numbers of adults were placed in two untreated cages. The dusted insects were kept in contact with 0·8 per cent. parathion dust throughout the experiment. After three hours 99 per cent. of the dusted individuals were moribund and 100 per cent. mortality was reached in seven hours; in the controls 1 per cent. mortality was recorded after 19 hours.

In a third laboratory experiment, about 250 adults were dusted at the rate of 30 kg./ha. with a 0·75 per cent. parathion dust and directly transferred to a clean rearing cage, with five of its six walls made of fly screen. Nearly 60 per cent. of the insects were moribund in three hours, and 100 per cent. mortality was recorded 12 hours after the dusting.

Field Trials.

The foregoing laboratory tests were sufficiently encouraging to warrant field tests and dusting was carried out in three different localities.

The results of the field experiments were evaluated by counting the numbers of *E. integriceps* taken per ten sweeps. The sweeps were made by the same individual throughout the experiments and a sweep net having a diameter of 45 cm. and a handle 175 cm. long was used. Every stroke of the net is termed a sweep and a count represents the total individuals taken and counted after ten sweeps of the net.

Experiment 1. 22.v.1950.

Two plots measuring 10×10 m., were dusted, one with 0·75 per cent. and the other with 0·8 per cent. pure parathion dust at an intended rate of 75 kg./ha. The plots were 8 m. apart, and the wind was blowing very strongly, so that the dust drifted some 25 m. and the real quantity per hectare could not be determined. Just after the dusting, two fly-screen cages, 2×2×2 m. in dimensions, each containing about 100 live nymphs, were placed each in the middle of the treated plots so as to increase the population therein. Six days later there were no living nymphs in the cages, and only two adults were taken in 12 counts in the two treated areas. Only ten nymphs were caught in 13 counts at a distance of 10 m. outside the treated area, and 57 nymphs in 29 counts at 20 m.

Experiment 2. 27.v.1950.

In another village three plots, each 20×100 m., were chosen, with an interval between the plots of at least 20 m. Each plot was dusted separately by means of a knapsack duster. Plot " A " was treated with 0·8 per cent. parathion dust at the rate of 17·5 kg./ha., but the dusting was very uneven. After 50 hours, 361 specimens were caught in ten counts on the treated plot, whilst an average of 501 were taken in each of ten counts (on either side of " A ") in the control plots. Plot " B " was dusted very satisfactorily with 0·75 per cent. parathion dust at the rate of 15 kg./ha. The results were recorded at the same time as in " A ", using the same technique; 498 specimens were taken in plot " B " against 610 in the control. Plot " C " was dusted with the same material and at the same rate as " A ", but the dusting this time was as good as that in " B "; the result was 314 in " C " compared with 471 in the control.

Experiment 3. 9.vi.50.

Finally a plot was chosen in a highly infested village. This plot measured 1,200 × 25 m., and a strip 1,200 × 8·33 m. was dusted with 48 kg. of 0·75 per cent. parathion dust, *i.e.*, 48 kg. /ha. The dusting was carried out with a 6 h.p. duster mounted on a Jeep fitted with a drag-sheet and the speed of the Jeep was so regulated as to cover the hectare with 48 kg. of the dust. The dusting was carried out in calm weather to avoid drift as far as possible. As many as five or six specimens per square metre were found dead in the treated area 60 hours later, while no dead could be found in the adjacent control. At the same time an equal number of sweeps was taken along each of two lines parallel to the edges of the field, equidistant from each other and from the two edges of the field ; 142 live individuals were collected in 40 counts taken along the whole length of the dusted plot, while 459 were taken in 40 counts similarly made in the adjacent control.

Undoubtedly many insects passed over from the harvested fields and the control to the dusted field, for at the time of recording (but not at the time of the dusting) all the wheat fields around the three-hectares plot of the experiment had been harvested, and the insects had all passed into the adult stage, when they could move freely to the only plot that had wheat still standing in it.

It is hoped to continue these experiments in the spring of 1951 with a view to finding the best concentration and rate of application of the dust, and the most efficient type of equipment.

Summary.

Eurygaster integriceps is a serious pest of wheat in Syria against which various methods of control have been tried.

Laboratory tests with a 0·8 per cent. parathion dust applied at the rate of 0·1 mg. /sq. cm. are described.

Three sets of field experiments were carried out with 0·75 per cent. and 0·8 per cent. parathion dust. Applied at a rate of 75 kg. /ha. these were very efficient but not economical. Applications of 0·8 per cent. dust with a knapsack duster at between 15 and 17·5 kg. /ha. gave about a 20 per cent. reduction in the infestation. A larger experiment in which 0·75 per cent. dust at a rate of 48 kg. /ha. was applied to a hectare of wheat, by means of a 6 h.p. duster mounted on a Jeep fitted with a drag sheet, gave a 70 per cent. control.

Acknowledgements.

The author expresses his sincere thanks to Mr. G. E. Jones of Plant Protection Ltd., Yalding, England, for supplying him by air with 50 kgs. of Fosferno (0·75 per cent. parathion dust) for the third experiment, and for his useful suggestions. He is also indebted to the Société des Usines Chimiques Rhône-Poulenc, Paris, for sending him 20 kgs. of their Rhôdiatox Poudrage containing 0·8 per cent. parathion.

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NEW SPECIES AND RECORDS OF *DIATRAEA* GUILD. FROM NORTHERN VENEZUELA (LEPID., PYRAL.).

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(Plate XIII.)

This paper is based for the most part on long series of moths (approximately 3,500 specimens) reared from known food-plants by the writer and his associates, in the course of intensive investigations on the sugar-cane moth borer (*Diatraea*) problem, carried out continuously since November 1946 for the Venezuelan Ministry of Agriculture. Some additional records, mostly from the collection of the Entomological Division of the Ministry, are included. It is hoped to publish later full ecological data as well as descriptions of the early stages, many of which were previously unknown.

The majority of the specimens here listed remain in the Entomological Division at Maracay, Venezuela, but representative series, and the types of the new forms, have been or will be presented to the British Museum (Natural History). Other specimens, including some paratypes, have been distributed to the Muséum National d'Histoire Naturelle (Paris), the United States National Museum (Washington), the New York Zoological Society (New York), the Department of Entomology of Cornell University (Ithaca), the Department of Entomology of the H.S.P.A. Experiment Station (Honolulu), the Imperial College of Tropical Agriculture (Trinidad), and other institutions.

The writer published recently (Box, 1948, pp. 32–39) a check-list of described species of *Diatraea* (*sens. str.*), with their geographical distribution, in which the following are recorded from Venezuela : *saccharalis* (F.), *lineolata* (Wlk.) (including the type), *impersonatella* (Wlk.) (including the type), *canella* Hamps., *minimifacta* Dyar, *pedibarbata* Dyar, *busckella busckella* Dyar & Heinr., *brunnescens* Box (including the type), *suffusella* Box, and *busckella rosa* Heinr. (including the type). More recently (Box, 1950c, p. 245) it has been possible to record *D. savannarum* Box, on the basis of one specimen (in the Department of Entomology, Cornell University) taken by R. Lichy in 1946 at Sanaripe, Amazonas Territory. With the exception of *minimifacta*, *suffusella* and *savannarum*, all of these have been found in the course of the present investigation, and are treated in the following text, together with three species, one subspecies and two forms, all new to science ; one previously recognised subspecies (*busckella rosa*) is here raised to specific rank. The total number of species now known in Venezuela, therefore, is 14, in one of which lower ranks are recognised. Three other species of *Diatraea, viz.*, *tabernella* Dyar, *bellifactella* Dyar and *albicrinella* Box, should occur in the country, but to date there are no positive records.

In the text that follows, described species (except *rosa* and *busckella*) are arranged chronologically, and the specimens examined are cited by localities within the respective Venezuelan States north of the Rio Orinoco* (see map, text fig. 2), listed generally in order from east to west. Place-names are those to be found on standard maps of the country ; altitudes (in metres) are approximate. In a territory as large as Venezuela, the citation of precise localities (including negative records in certain cases) is absolutely essential for the correct interpretation of the geographical and ecological distribution of a species, and for this reason they are here presented in complete form, though condensed as far as possible.

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*A few records from Upata, 110 kilometres east of Ciudad Bolivar, and south of the Rio Orinoco, are included for convenience, although this region properly pertains to southern Venezuela.

It is not considered necessary to give the dates of collection except in special instances, since the material examined consists of moths reared from larvae taken in more or less permanent breeding places, where they have been or could have been found throughout the period of the investigation.

The greater part of the collection was made by the writer personally with the collaboration of Pietro Guagliumi, P. Paredes Ramirez, J. Ledezma Turmero and F. Mejías, of the Section of Sugar-cane Investigations. Other collections during the same period have been made on our behalf by P. Fenjves, F. Fernández Yépez, L. A. Salas and W. Szumkowski, respectively in charge of other sections of the Entomological Division, and A. Ciccarone, of the Phytopathological Division. To all who have thus helped with the work the writer takes this opportunity to express his very sincere thanks for their valued assistance.

Once again it is a pleasure to acknowledge the help and advice of Mr. W. H. T. Tams, of the British Museum (Natural History), with whom the writer discussed several points of taxonomy during his visit to London in 1948. It is indeed due to the excellent photomicrographs of the genitalia of specimens belonging to the *rosabusckella* group made by Mr. Tams, that the writer has been able to recognise the status of these moths here adopted. Thanks are also due to Dr. E. A. Chapin and Mr. Carl Heinrich for being so kind as to send on loan from the United States National Museum slides and photographs which proved indispensable to the present study.

Pl. XIII, figs. 1–4 and 9–14, all inclusive, are from photographs by Mr. W. H. T. Tams; the others are by the author.

DIATRAEA Guild.

Diatraea Guilding, 1828, Trans. Soc. Encour. Arts, **46,** p. 148.

Diatraea saccharalis (**F.**).

Phalaena saccharalis Fabricius, 1794, Ent. Syst., **3** (2) p. 238; 1794, Skrift. af Naturh.-Selsk., Copenhagen, **3** (2), p. 64, Pl. vii, fig. 1.

Diatraea saccharalis (F.) Comstock, 1881, U.S. Dep. Agric., Ent. Rep. 1880, p. 240.

Except where otherwise stated, the following records are of specimens reared from larvae in sugar-cane :—

370 ♂♂, 440 ♀♀. TERRITORIO DELTA AMACURO: Araguaymujo, Lower Orinoco (near sea-level), in rice (with *Rupela* sp.), v.1948 (*A. Ciccarone*). BOLIVAR: Upata (300 m.), in maize (with *lineolata* and *rosa*). MONAGAS: near Uracoa, Lower Orinoco (sea-level), at light, v.1947 (*L. A. Salas*); San Francisco (400 m.), San Antonio de Maturin (440 m.) (with *rosa*), Guanaguana (420 m.) (with *canella* and *rosa*). SUCRE: Irapa (near sea-level), iv–v.1944 (*F. Fernández Yépez*); Cumanacoa (230 m.) (very local, with *canella*, *impersonatella* or *rosa*). ANZOATEGUI: north bank of Rio Orinoco, La Soledad (30 m.), iv–v.1944 (*F. F. Y.*). GUARICO: Altagracia del Orituco (300–400 m.) (with *canella*); Calabozo (100 m.) (with *rosa*). MIRANDA: Paparo (near sea-level), in rice, i–ii.1942 (*F. de la Madriz & P. Sanojo*); Guatire (325 m.), Guarenas (385 m.), Petare (850 m.) (with *canella* and *rosa*); Santa Lucia (180 m.) (with *canella* and *rosa*); Cua (250 m.), in maize (with *lineolata*); Las Adjuntas (920 m.). ARAGUA: Las Tejerias, El Consejo, La Urbina (500–550 m.), La Victoria, San Mateo, Cagua, Turmero, Maracay and E. side of Lake Valencia (450–525 m.) (with *canella* and *rosa*), and in maize (with *lineolata* and *rosa*); Guayabita, near Turmero (500 m.), in *Hymenachne amplexicaulis*, *Panicum purpurascens* and *Paspalum fasciculatum*, bordering a stream in cultivated area; Cagua (450 m.), in *P. purpurascens*, viii–ix. 1949; Gonzalito, near Maracay (450 m.), in millet (*Sorghum vulgare*) in genetical experiment plots, ii.1948 (*A. Ciccarone & H. E. B.*), and in wheat (*Triticum aestivum*) (with *lineolata*), and barley (*Hordeum vulgare*), v.1949; El Limon, near Maracay

(500 m.) in *Coix lachryma-jobi* ; Villa de Cura (530 m.) (with *rosa*), and in maize (with *lineolata* and *rosa*) ; Ocumare de la Costa (sea level), in *Panicum grande*, in river estuary, and in *Paspalum virgatum* in waste lands nearby, also in Uba Cane (cultivated for forage) and *Oryza latifolia* (with *Rupela* sp.) in roadside trench in cacao plantation. CARABOBO : N. side of Lake Valencia, Hacienda Cura, near San Joaquin (450 m.) (with *canella* and *rosa*), and in maize (with *lineolata* and *rosa*) and *Panicum maximum* ; W. side of Lake Valencia, Central Tacarigua (450 m.), in maize (with *lineolata* and *rosa*) ; La Encantada, near Tacarigua (550 m.) and riversides on Carabobo savannahs (450–500 m.), in *Panicum grande* ; Moron (sea-level), in maize (with *lineolata*), in *Hymenachne amplexicaulis* at back of mangrove swamp, and in *Panicum purpurascens* ; between Moron and Boca del Yaracuy (near sea-level), in *Panicum grande* (with occasional *pedibarbata*), ix.1949 ; Urama (30 m.), in *Paspalum fasciculatum* (with *impersonatella*), also in *P. purpurascens* and *Cyperus ligularis*, on edge of wet-season pond in secondary forest, iv.1948, and in *Oryza latifolia* (with *Rupela* sp.) in roadside ditches in coastal evergreen forest. COJEDES : San Carlos (150 m.), in maize (with *lineolata*). YARACUY : Nirgua (800 m.) and upper Rio Nirgua valley (500–600 m.) (with *rosa*, and locally with *canella*) ; Bella Vista and Maporito (20–35 m.), in *P. grande* and *O. latifolia* (with *Rupela* sp.), in roadside trenches in coastal evergreen forest ; Marin (125 m.) (with *canella*) ; San Felipe (200 m.), also in rice, ii–iii.1943 (*S. B. & C. H. Ballou*) ; Rio Yaracuy, near San Felipe, also in *P. grande* and *Panicum maximum* ; Chivacoa (230 m.), Yaritagua (400 m.) (with *b. busckella* and *canella*). FALCON : no records. LARA : Rio Turbio valley around Barquisimeto (350–450 m.), El Tocuyo valley (600–750 m.) (with *b. busckella*, *canella*, and at El Tocuyo also with *impersonatella*), and in maize (with *lineolata*), and in *Paspalum fasciculatum* (with *canella* and *impersonatella*) by side of Rio Tocuyo, iv.1948 ; Humocaro Bajo (900 m.) (with *b. busckella*, *canella* and *impersonatella*) ; Sicarigua (475 m.) (with *b. busckella*). PORTUGUESA : Guanare (180 m.) (with *canella*, in newly established canefields in cleared deciduous forest), ii.1949 ; Biscucuy (700 m.) (with *lineolata*). BARINAS : near Barinas (150 m.) (with *canella*) ; Barinitas (600 m.) ; Puerto Nutrias (Rio Apure), in *Paspalum fasciculatum* (rare), i.1950. APURE : San Fernando de Apure (70 m.), in *P. fasciculatum* on river banks at edge of town ; Guasdualito-El Amparo road, Upper Apure (180 m.), also in rice and *Oryza latifolia*, ii.1950. TRUJILLO : no records. MERIDA : Mérida (1,640 m.), in maize, vii.1938 (*A. P. Troconis*) ; Rio Chama valley, Egido to Mérida (1,100–1,700 m.) (locally with *b. busckella*) ; Palomitas, El Vigia road (600 m.) (with *b. busckella*) ; Tovar (970 m.), Bailadores (1,750 m.). TACHIRA : La Grita (1,440 m.), La Quinta (1,225 m.) ; San Cristobal (850 m.), Santa Ana (700 m.), La Esterlina, Rio Quinimarí (750 m.), Rubio (825 m.) (with *b. busckella* and *canella*) ; Ureña, Rio Táchira, on Venezuela-Colombia frontier (310 m.) (with *b. busckella*), and in maize (with *lineolata*). ZULIA : no records.

The long series of specimens of both sexes shows great variation in the size of individuals (expanse of males 18·0–26·5 mm., of females 23·0–38·5 mm.), and includes colour variants which range from pale straw, sometimes tinged with yellowish, to brownish. Small brownish specimens tend to be more frequent among moths reared from larvae in millet, wheat, and some of the aquatic grasses, than among those from sugar-cane or maize. It has been noted that pupae developing in water grasses (*Hymenachne amplexicaulis*, *Panicum grande*, *Oryza latifolia*) have the abdominal spines conspicuously longer than in those from cultivated plants. This may be a primitive adaptation which enables the pupa to wriggle rapidly up the larval burrow and thus save itself from drowning when the plant is inundated, and which has become modified when the species has bred for generations in a more terrestrial habitat.

Of special interest is the finding of *D. saccharalis* in a sedge. A nearly full-grown larva was found low down in its tunnel in a stalk of *Cyperus ligularis*, in a locality where the borer was extremely rare (only one other larva was found here, in *Panicum* *purpurascens*). The only other record of this species feeding in one of the Cyperaceae appears to be that of Cleare (1939, p. 99) who found larvae (parasitised by *Metagonistylum minense* Ts.) in *Cyperus articulatus* on the coastlands of British Guiana.

Our records of *saccharalis* attacking wheat (in company with *lineolata*) are confined to observations made at the Genetics Experimental Station at Gonzalito, Aragua State, and there is no evidence that the wheat cultivated on a large scale in the Andean region (Trujillo and Mérida) is attacked by this species. Dr. Johannes Wille, however, informed the writer that wheat is quite commonly attacked by this borer on the slopes of the Andes in Peru (Wille, 1932).

D. saccharalis ranges throughout most parts of northern Venezuela where its food-plants occur, but nevertheless its absence from certain regions is noteworthy. No records are forthcoming from Trujillo State, in spite of considerable research in sugar-cane, maize and wild grasses, although the general ecological conditions differ in no marked respect from those of adjacent States where the insect occurs in abundance ; neither was this borer found in any of these plants in the Andean region north-east of the Mucuchies Pass, which (at 4,000 metres altitude) separates the Motatan and Chama valleys. South-west of this watershed, however, in the Chama valley from above Mérida (1,750 m.) southwards, *D. saccharalis* is fairly common in sugar-cane. It is a curious fact that *saccharalis* has not been found among the several thousand hectares of sugar-cane at Central Tacarigua (Carabobo), where *rosa* and *canella* are the dominant borers, yet *saccharalis* occurs there in maize and in certain aquatic grasses nearby, and in sugar-cane at the Hacienda Cura, less than 20 kilometres distant and in the same ecological zone. At Urama, on the coastal region west of Puerto Cabello, the only borer in sugar-cane appears to be *rosa*, in spite of apparently ideal conditions for *saccharalis*, which breeds regularly in aquatic grasses in nearby swamps and roadside trenches.

Diatraea lineolata (Wlk.).

Leucania lineolata Walker, 1856, List Lep. Ins. Brit. Mus., **9,** p. 100.

Diatraea lineolata (Wlk.) Hampson, 1895, Proc. zool. Soc. Lond., **1895,** p. 953.

This species was described from " Venezuela " with no further indication of its origin. The type locality is therefore designated as Maracay, Aragua State, Venezuela.

Except where otherwise stated, the following records are of specimens reared from larvae in maize :—

200 ♂♂, 213 ♀♀. Bolivar : Upata (300 m.) (with *rosa* and *saccharalis*). Monagas : Caripe (900 m.), Aragua de Maturin (220 m.). Sucre : Cumanacoa (230 m.). Miranda : Caucagua (60 m.), ii.1948 (*F. F. Y.*) ; Charallave (300 m.) ; Cua (250 m.) (with *saccharalis*). Guarico : Calabozo (100 m.) ; San Juan de los Morros (420 m.). Aragua : Guiripa, near San Casimiro (500 m.), vi.1949 (*F. F. Y.*) ; El Consejo (550 m.) (with *rosa* and *saccharalis*) ; San Mateo, Cagua, Turmero, Maracay, and E. side of Lake Valencia (450–525 m.) (with *rosa* and *saccharalis*) ; Gonzalito, near Maracay (450 m.), in wheat (*Triticum aestivum*) (with *saccharalis*), iv–v.1949, and nearby in Guatemala Grass (*Tripsacum laxum*) (with *rosa*) ; S.E. of Lake Valencia, near Magdaleno (430 m.) ; Villa de Cura (550 m.) (with *rosa* and *saccharalis*), and in Broom-corn (*Sorghum vulgare* var.). Carabobo : N. side of Lake Valencia, La Cabrera and San Joaquin, W. side of Lake Valencia, Central Tacarigua (430–450 m.) (with *rosa* and *saccharalis*) ; S. side of Lake Valencia, near Guigue (with *rosa*) ; Carabobo savannahs (500 m.), Bejuma (600 m.) ; Moron (near sea-level) (with *saccharalis*) ; Guaremal, near Urama (30 m.). Cojedes : San Carlos (175 m.) and San Rafael (150 m.) (with *saccharalis*). Yaracuy : Nirgua (800 m.) ; Marin, near

San Felipe (125 m.), in peasant's sugar-canes,* xi.1947 ; San Felipe (225 m.) ; San Pablo and Guama (250–300 m.). FALCON : Boca del Aroa (sea-level). LARA : Rio Turbio Valley near Barquisimeto (375 m.) ; Quibor (500 m.) ; El Tocuyo valley (600–750 m.) (with *saccharalis*) ; Humucaro Bajo (900 m.). PORTUGUESA : Camaguapo, near Acarigua (180 m.) ; near Chabasquen (900 m.), in *Tripsacum laxum* cultivated in peasant's plot in a protected ravine ; Biscucuy (700 m.), one larva in sugar-cane (with *saccharalis*), vi.1947. APURE : Birhuaca, near San Fernando de Apure (70 m.) ; San Vicente, near Bruzual (100 m.). TRUJILLO : Mendoza Fria (950 m.), one larva in *Tripsacum australe* (with one *b. busckella*) on roadside embankment in cultivated area, iv.1948 ; El Cenizo (near sea-level), 28.viii.1950 (*P. Fenjves*). MERIDA : Mérida (1,640 m.), xii.1938 (*J. M. Viloria*). TACHIRA : San Cristobal and Táriba (800–850 m.), Santa Ana (700 m.) ; Ureña, Rio Táchira, on Venezuela-Colombia frontier (310 m.) (with *saccharalis*).

The whole series is very constant in coloration, and with little variation in size among specimens reared from maize ; those reared from larvae in wheat, however, are much smaller (expanse of males 19·5–21·0 mm., of females 22·0–23·5 mm.).

Sugar-cane, wheat and *Sorghum* are food-plants not previously recorded for this species, which was hitherto known, from published records, only in maize and teosinte (*Euchlaena mexicana*) and *Tripsacum* spp., all of which belong to the tribe Tripsaceae of the family Gramineae. The occurrence in sugar-cane is interesting, but is regarded as accidental. *D. lineolata* still remains unknown in any true wild grass host.

Diatraea impersonatella (Wlk.).

Crambus impersonatellus Walker, 1863, List Lep. Ins. Brit. Mus., **27,** p. 163.

Diatraea impersonatella (Wlk.) Box, 1931, Bull. ent. Res., **22,** p. 41, pl. iii, figs. 2, 3.

The species was described from seven specimens, five from Venezuela and two from Santarem. Hampson labelled as type one of the Venezuelan specimens in the British Museum, which bears no further indication of its origin. The type locality is therefore designated as Cumaná, Sucre State, Venezuela.

59 ♂♂, 52 ♀♀. SUCRE : Cumaná (near sea-level), in sugar-cane ; Cumanacoa (230 m.), in sugar-cane (with *canella*, *rosa*, or locally with *saccharalis*). MIRANDA: Rio Tuy at Cua (250 m.), in *Paspalum fasciculatum* by riverside, iii.1948. CARABOBO : Urama (10 m.) to Guaremal (30 m.), in *P. fasciculatum* (with *saccharalis*) and *P. virgatum* (with *canella*) by roadside in coastal evergreen and secondary forests. YARACUY : Bella Vista (30 m.), in *P. virgatum* (with *canella*) ; Guayabo (10 m.), Banana Co.'s lands, males abundant at light, probably developing from larvae in *P. virgatum*, 29.iii.1949 (*F. Fernández Yépez & P. Fenjves*). LARA : El Tocuyo valley (600–750 m.), Humocaro Bajo (900 m.), in sugar-cane (with *b. busckella*, *canella* and *saccharalis*), also in *P. fasciculatum* (with *canella* and *saccharalis*) by side of Rio Tocuyo (575 m.), iv.1948. TRUJILLO : Betijoque (600 m.), in *P. virgatum* in second-growth bushlands on hillsides (one larva, with many *canella*), iv.1948. APURE : San Fernando de Apure (70 m.), in *P. fasciculatum* (rare), i.1950 ; Guasdualito—El Amparo road, Upper Apure (180 m.), in *P. virgatum* (with *canella*).

Although *D. impersonatella* is extensively distributed in northern Venezuela, it has developed as a sugar-cane borer only in a few restricted localities in the widely separated States of Sucre and Lara. It would seem that we have here an example of a species in process of adapting itself gradually from wild to cultivated host-plants, and one which may in time become a widespread and possibly serious pest of sugar-cane in Venezuela and other parts of South America, as it is today in Trinidad.

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*The plot consisted of less than a hundred plants of sugar-cane, scattered among cassava, banana, etc. Considerable search yielded one larva and one pupa of *Diatraea*, from which *lineolata* were reared.

Diatraea canella Hamps.

Diatraea canella Hampson, 1895, Ann. Mag. nat. Hist., (6) **16,** p. 349.

Except where otherwise stated, the following records are of specimens reared from larvae in sugar-cane :—

192 ♂♂, 224 ♀♀. MONAGAS : Guanaguana (420 m.) (with *rosa* and *saccharalis*). SUCRE : Cumanacoa (230 m.) (with *impersonatella, rosa* or locally with *saccharalis*). GUARICO : Altagracia del Orituco (350–400 m.) (with *saccharalis*). MIRANDA : Guatire (325 m.), Guarenas (385 m.), Petare (850 m.), Santa Lucia (180 m.) (with *rosa* and *saccharalis*) ; Ocumare del Tuy (200 m.) Cua, (500 m.) (with *rosa*). ARAGUA : Las Tejerias, El Consejo, La Urbina, La Victoria, San Mateo, Turmero, Maracay (450–525 m.) (with *rosa* and *saccharalis*), also in *Setaria paniculifera* (one larva only) under shade of trees at El Consejo, in *Cyperus* sp. (one larva only) in canefield at La Urbina, 2.viii.1950, and in *Paspalum virgatum* near Maracay, 18.viii.1950. CARABOBO : N. side of Lake Valencia, Hacienda Cura, near San Joaquin (450 m.) (with *rosa* and *saccharalis*) ; W. side of Lake Valencia, Central Tacarigua (430 m.) (with *rosa*), and in maize (one larva with numerous *lineolata* and *rosa*), xii.1948 ; Carabobo savannahs (450–550 m.), in *Andropogon bicornis* (with *Rupela* sp.) ; Guaremal, near Urama (20 m.), in *Paspalum virgatum* (with *impersonatella*) by roadside in coastal evergreen forest. YARACUY : Upper Rio Nirgua valley at Los Cogollos (600 m.) (with *rosa* and *saccharalis*) ; Guayabo (10 m.), Banana Co.'s lands, in *P. virgatum* ; Marin, near San Felipe (125 m.) (with *saccharalis*) ; San Pablo (400 m.) (with *b. busckella*) ; Chivacoa (230 m.), Urachiche (400 m.), Yaritagua (400 m.) (with *b. busckella* and *saccharalis*). LARA : Rio Turbio valley around Barquisimeto (350–450 m.) (with *b. busckella* and *saccharalis*), and in *P. virgatum* around canefields ; Sarare (250 m.) ; El Tocuyo valley (600–750 m.), Humocaro Bajo (900 m.) (with *b. busckella, impersonatella* and *saccharalis*) also in *Paspalum fasciculatum* by side of Rio Tocuyo (with *impersonatella* and *saccharalis*), iv.1948. PORTUGUESA : Guanare (180 m.), (with *saccharalis*) in newly established canefields in cleared deciduous forest, ii.1949. BARINAS : near Barinas (150 m.) with *saccharalis*. APURE : Birhuaca, near San Fernando de Apure (70 m.) ; San Vicente, near Bruzual (100 m.), in *Andropogon bicornis* (with *Rupela* sp.) in fluvial savannahs i. & iii.1950, Guasdualito—El Amparo road, Upper Apure (180 m.), also in *P. virgatum* (locally with *impersonatella*), by roadsides and around canefields. TRUJILLO : near Carache (575 m.) (with *b. busckella*) ; San Pedro de Betijoque (750 m.) (with *b. busckella*) ; Betijoque (600 m.), in *P. virgatum* (with *impersonatella*) and larval remains in old borings in *A. bicornis* in second-growth bushlands on hillside, iv.1948 ; Rio Momboy valley, above Valera (700 m.) and Mendoza Fria (960 m.) (with *b. busckella*). MERIDA : no records. TACHIRA : San Cristobal (850 m.), La Esterlina, Rio Quinimarí (750 m.), Rubio (825 m.) (with *b. busckella* and *saccharalis*). ZULIA : no records.

The above, in conjunction with previous published records, indicate *D. canella* to be widespread in northern Venezuela, with an altitudinal range from sea-level to near 1,000 metres. The discovery of this species in Táchira State, and on the Upper Rio Apure, in both cases within a few kilometres of the frontier with Colombia, is especially interesting, because hitherto *canella* has been regarded as being more or less confined to the eastern and north central regions, in Venezuela. It is probable that the original food-plant in this country is the savannah grass, *Andropogon bicornis*, whence it has spread into the cultivated areas. The solitary instance of this borer feeding in maize may be considered as accidental, as also those in *Setaria* and *Cyperus*, which have not previously been recorded as food-plants.

The basic colour of the body and fore-wing in both sexes varies from greyish to pinkish, and there is considerable variation in the size of the discocellular dot. Specimens reared from larvae in *A. bicornis* are small (expanse of males 20·0–21·5 mm., of females 25·0–27·5 mm.).

Diatraea pedibarbata Dyar.

Diatraea pedibarbata Dyar, 1911, Ent. News, **22,** p. 202.

21 ♂♂, 25 ♀♀. YARACUY : Mouth of Rio Yaracuy, close to the seashore, in *Typha angustifolia,* ii–iii.1948 ; near above locality, in *Panicum grande* (with *saccharalis*) in freshwater swamps near the coast, xi.1949 ; Marin, near San Felipe (125 m.), in *T. angustifolia* in swampy ground on edge of a pasture, iv–v.1948. FALCON : Mouth of Rio Aroa (sea-level), in *T. angustifolia,* xi.1949. TRUJILLO : Rio Jimenez, near Pampanito (350 m.), in *Panicum grande,* xii.1946, iii.1947 & iv.1948 ; La Ceiba, on east shore of Lake Maracaibo, in *P. grande* on edge of coastal swamp-forest, iv.1948.

A species not previously recorded from Venezuela.* There are no obvious differences between moths reared from *Typha* and those from *Panicum grande,* but there is considerable variation in size among them all (expanse of males, 21·5–29·0 mm., of females 27·0–39·5 mm.).

The *Typha* is also bored by the large larvae of an Agrotid which Mr. Tams thinks may be new, and also by those of certain weevils of the genus *Cholus* Germ. *D. pedibarbata* has not been found in the extensive beds of *Typha* (the " cat-tail, *Typha tenuifolia* ", of von Humboldt) which are a characteristic feature of the shores of Lake Valencia (Carabobo and Aragua States).

Diatraea brunnescens Box.

Diatraea brunnescens Box, 1931, Bull. ent. Res., **22,** p. 29, pl. v, fig. 1 ; 1935, *op. cit.,* **26,** p. 326.

Diatraea incertella Box, 1931, *op. cit.,* **22,** p. 30, pl. 1, figs. 16, 17.

15 ♂♂, 22 ♀♀. ARAGUA : Aragua National Park at Guamita (Maracay–Rancho Grande road, 750–800 m.), in *Pennisetum bambusiforme* on roadside embankment in montane forest, rare, xii.1947, ix.1948 ; Maracay (450 m.), 1♀ at light in house, 23.vii.1950. CARABOBO : Upper Rio Chirgua valley, near Bejuma (700 m.), in *P. bambusiforme* in bushlands on deforested hillsides, ii–xi.1948. APURE : Guasdualito—El Amparo road, Upper Apure (180 m.), in *Setaria vulpiseta* (frequent, with occasional *D. silvicola* sp.n.) and *S. paniculifera* (rare), on edge of forest and in nearby clearings, ii.1950.

In size, the above specimens are variable and, especially those reared from *Setaria,* tend to be larger than the type series from Ciudad Bolivar (Rio Orinoco), the expanse of males reaching 22·0 mm., and of females 27·5 mm. In the fore wing, veins 4 and 5 may be separate or united at their bases ; in the hind wing these veins are united.

This species was described from Ciudad Bolivar (Rio Orinoco), where conditions of the habitat would appear to be very different from those of the upland localities cited above. The latter, however, are in the vicinity of induced *Trachypogon-Curatella* savannahs, which is the type of country where the late Dr. J. G. Myers discovered the larvae of *D. brunnescens* feeding in *Setaria vulpiseta* on the Kanuku mountains, in the hinterland of British Guiana.

Diatraea rosa Heinr., new status (figs. 1, 2 ; Plate XIII, figs. 1, 2).

Diatraea busckella var. *rosa* Heinrich, 1931, Proc. U.S. nat. Mus., **79,** no. 2879, p. 4 ; Box, 1935, Bull. ent. Res., **26,** p. 330.

Diatraea busckella (nec Dyar & Heinr., 1927, Proc. U.S. nat. Mus., **71,** no. 2691, p. 16, figs. 5, 53,=*D. busckella busckella* Dyar & Heinr.) Box, 1931, Bull. ent. Res., **22,** p. 43 (in part and so far as concerns Venezuelan records) pl. iii, fig. 5.

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* *D. pedibarbata* was erroneously referred to as " Sp. nov. A " by the writer (Box, 1947).

Diatraea busckella subsp. *rosa* (Heinr.) Box 1948, Bol. Ent. venez., **7,** p. 39.

D. rosa Heinr. is differentiated specifically from the related *D. busckella* Dyar & Heinr., on the evidence provided by the unique colour of the body and fore wing in both sexes, and the distinctive characters of the male and female genitalia. The moths attain a larger size than in the various forms of *busckella* recognised below, the expanse of the males of *rosa* often reaching 33·0 mm., and of the females 42·0 mm. (see note on page 393).

Heinrich described *D. busckella* var. *rosa* as " A Venezuelan race agreeing with typical *busckella* in genitalia ; but with a distinctly pinkish ocherous tint on fore wings of both sexes. Typical *busckella* is pale straw color." The present writer (Box, 1931) misidentified Venezuelan moths as *busckella*, which was known to him at the time only by description. He drew attention, however, to the fact that all of the specimens before him had " the fore wing of a most distinctive colour, not seen elsewhere in *Diatraea*, viz. capucine buff to light ochraceous salmon, tinged with pinkish cinnamon " (Ridgeway)*. It seems that Heinrich did not make a critical comparison of the genitalia of *rosa* and *busckella*, and his statement that they agreed with each other was accepted by the writer in his later (Box, 1935b) discussion of this moth. Our present studies show that these organs, while of similar general construction—to be expected in two closely related moths—differ in several characters which appear to be quite constant in each species (fig. 1). In the male, attention is drawn to the proportions of the uncus and gnathos, the position and shape of the lateral lobe of the tegumen, the form of the knob-like spiny projection at the base of the harpes, and the aedeagus ; in the female, to the shape of the raised papillose-setose chitinised lobes in front of the genital opening. The figures of *rosa* are based on topotypical specimens, from Carabobo.

Except where otherwise stated, the following records are of specimens reared from larvae in sugar-cane :—

470 ♂♂, 300 ♀♀. Bolivar : Upata (300 m.), and in maize (with *lineolata* and *saccharalis*). Monagas : Lower Orinoco, near Tucupita (sea-level), iv.1948 (*L. A. Salas*) ; Aragua de Maturin (220 m.), Caicara (230 m.), San Antonio de Maturin (440 m.), San Francisco (410 m.) (locally with *saccharalis*), Guanaguana (420 m.) (with *canella* and *saccharalis*). Sucre : Cumanacoa (230 m.) (with *canella*, *impersonatella* and locally with *saccharalis*). Anzoategui : Barcelona (near sea-level), Santa Inez (180 m.). Guarico : Llanos del Guárico, west of Pariaguan (220 m.) ; Calabozo (100 m.) (with *saccharalis*). Miranda : Guatire (325 m.), Guarenas (385 m.), Petare (850 m.), Santa Lucia (180 m.) (with *canella* and *saccharalis*) ; Ocumare del Tuy (200 m.) ; near Cua (250 m.) (with *canella*). Aragua : San Casimiro (500 m.), Guiripa (500 m.) ; Las Tejerias, El Consejo, La Urbina (500–550 m.) (with *canella* and *saccharalis*), and in maize (with *lineolata* and *saccharalis*) ; La Victoria, Cagua, San Mateo, Turmero, Maracay, El Limon, and E. side of Lake Valencia (450–525 m.) (with *canella* and *saccharalis*), and locally in maize (with *lineolata* and *saccharalis*) and *Tripsacum laxum* (with *lineolata*) ; near Choroní (350 m.) ; Villa de Cura (550 m.) (with *saccharalis*), and in maize (with *lineolata* and *saccharalis*). Carabobo : N. side of Lake Valencia, Hacienda Cura, near San Joaquin (450 m.) (with *canella* and *saccharalis*), and in maize (with *lineolata* and *saccharalis*) ; S. side of Lake Valencia, near Guigue (430 m.), in maize (with *lineolata*) ; W. side of Lake Valencia, Central Tacarigua (430 m.) (with *canella*), and in maize (with *lineolata* and *saccharalis*) ; Guaiguaza, near Puerto Cabello (150 m.) ; Central Lucinda, near Urama (near sea-level) ; Bejuma, Montalban, Miranda (600–700 m.) ; Aguirre, at light, xi.1947 (*F. Fernández Yépez*). Yaracuy : Nirgua (750 m.) (with *saccharalis*), and in maize (with *lineolata*) ; Upper Rio Nirgua valley (550–650 m.) (with *canella* and *saccharalis*). Falcon : Capadare (200 m.),

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*The fine pinkish tint of fresh specimens tends to fade with age, but nevertheless the colour of *rosa* remains quite distinct from that of *busckella*.

Mirimire (300 m.). COJEDES : Tinaco (260 m.) ; San Carlos (150 m.). PORTUGUESA : Araure (230 m.). APURE : Birhuaca, near San Fernando de Apure (70 m.) (with *canella*) ; Santa Rosa, near Achagua ; San Vicente, near Bruzual (Rio Apure) (100 m.) ; Guasdualito (180 m.) (with *canella*).

The various records of Myers (1932, pp. 265–268 ; 1935a, pp. 224–225 ; 1935b, pp. 337–339) citing " *busckella* " from several localities in northern Venezuela, including the Apure savannahs, apply actually to the present species, as also his reference (1935c, p. 225) to the larvae of " *busckella* " in a mixed infestation, with those of *saccharalis* and *impersonatella* " in the high grass savannah bordering the Rivers Apure and Portuguesa, the host being *Paspalum fasciculatum* ." This last-mentioned record is of special interest and importance, because it is the only instance where the larvae of *rosa* have been found in a wild habitat. In the course of the present investigation, we have not succeeded in finding larvae of *rosa* in *P. fasciculatum* or other wild hosts, in spite of constant and diligent search, which has included Myers' locality on the Apure and Portuguesa rivers.

D. rosa appears to be confined to Venezuela. In a recent paper* (Box 1950b, p.44, fig. 2), the writer has briefly discussed the geographical distribution of this species in relation to that of *D. busckella busckella* (see Map, fig. 2) and the subject has been dealt with at length in the writer's recently issued progress report (Box, 1950a).

A paper has recently been published by P. Fenjves (1950), in which the distribution of *D. busckella busckella* and *D.* " *busckella* " *rosa* is treated in some detail.

The larva of *D. rosa* is by far the most formidable of the various sugar-cane borers in Venezuela. Its presence in the extreme east of Venezuela, close to the seaboard, is to be regarded as a constant menace to the sugar estates of Trinidad.

Diatraea busckella Dyar & Heinr. (figs. 1, 2 ; Plate XIII, figs. 3, 4 & 12).

Diatraea busckella Dyar & Heinrich, 1927, Proc. U.S. nat. Mus., **71,** no. 2691, p. 16, figs. 5, 53 ; Box, 1931, Bull. ent. Res., **22,** p. 43 (in part ; excluding pl. iii, fig. 5, and all references to Venezuelan specimens = *D. rosa* Heinr.) ; 1935, Bull. ent. Res., **26,** p. 329.

Differs from *D. rosa* Heinr., in the colour of the fore wing in both sexes, which varies from pale ochreous (light buff) to brownish, sometimes stramineous, never with a pinkish tinge, and in the characters of the male and female genitalia (fig. 1) (see note on page 393).

The species is regarded as polytypic, for it has been noted that there is considerable variation in the depth of the ochreous or brownish colour, especially in the degree of contrast in the lines and shaded areas which form the wing pattern, and some of these variants appear to be quite constant in populations from different localities and food-plants. One of them, described below as subsp. *setariae* nov., also has certain constant characters in the male genitalia.

Through the kindness of Mr. Carl Heinrich, the writer has received photographs of the genitalia of the male type of *D. busckella* (from Porto Bello, Panama), and slides were sent on loan from the United States National Museum of the genitalia of paratype females (also from Panama). With the aid of this important material, as well as certain photomicrographs which Mr. Tams kindly made in 1948 from Venezuelan specimens now in the British Museum, it has been possible not only to differentiate *rosa* from *busckella*, but also to recognise in the latter certain forms which include the new subspecies described below. Representative samples of *busckella* from different

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*Since that paper was submitted for publication, *D. rosa* has been found at several localities in Apure State, as far west as Guasdualito and also in Falcon State, but there is still no overlapping in the known ranges of *rosa* and *busckella busckella*, except for the presence of a local form of the latter at Ocumare de la Costa (Aragua State) ; see map, fig. 2.

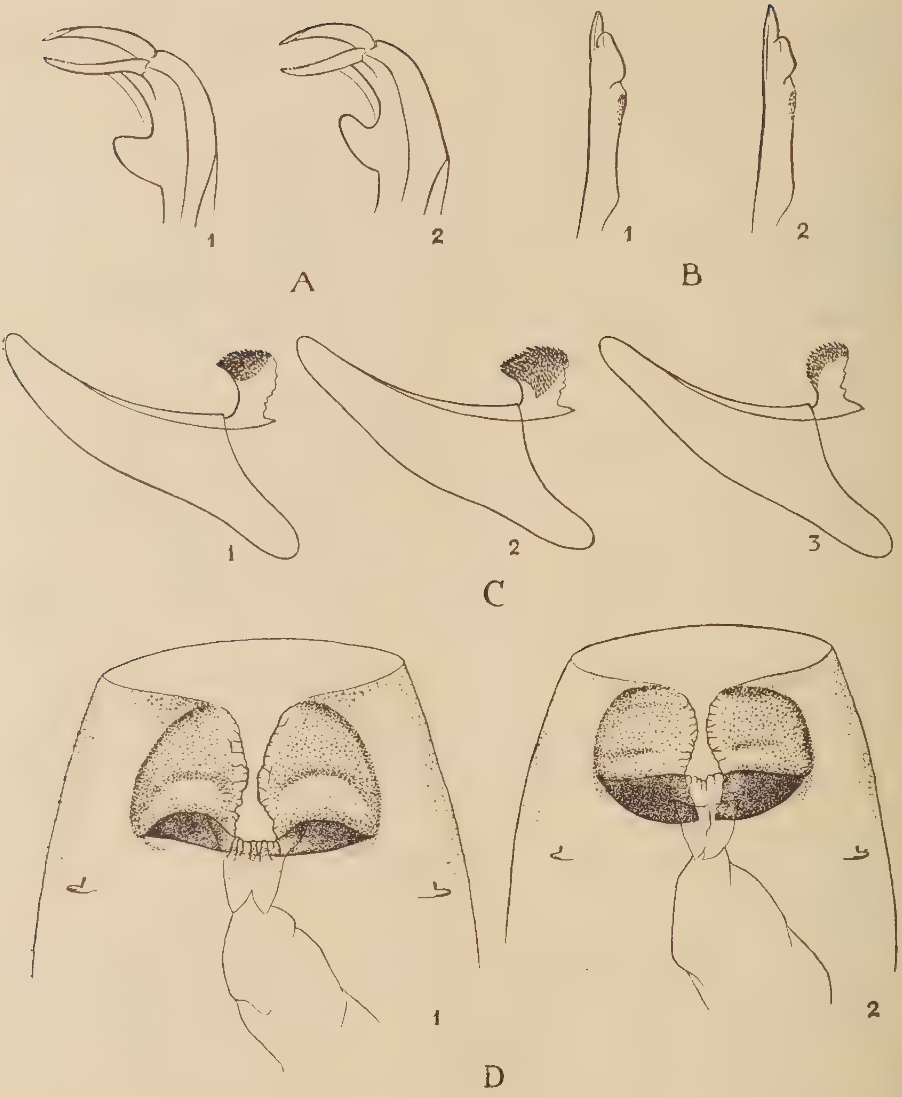

Fig. 1.—Genitalia of *Diatraea rosa* Heinr. and *D. busckella* Dyar & Heinr. (diagrammatic). (*a*) ♂, Tegumen, uncus and gnathos : (1) *D. rosa* ; (2) *D. busckella*. (*b*) ♂, Aedeagus : (1) *D. rosa* ; (2) *D. busckella*. (*c*) ♂, Harpes : (1) *D. rosa* ; (2) *D. busckella busckella* ; (3) *D. b.* subsp. *setariae* n. (*d*) ♀, Genital opening and part of bursa copulatrix ; (1) *D. rosa* ; (2) *D. busckella*.

localities and food-plants in Venezuela have been dissected, and the male genitalia prove to have reliable characters for identification. These organs (and those of the female) readily permit the separation of the various forms of *busckella* from *rosa* (fig. 1, *a–d*), and the male harpes enables typical *busckella* to be separated from *b.* subsp. *setariae* nov. (fig. 1, *c*2, 3).

Diatraea busckella busckella subsp. typ. (figs. 1, 2).

Diatraea busckella subsp. *busckella* (Dyar & Heinr., 1927), Box, 1948, Bol. Ent. venez., **7,** p. 36.

Forma *busckella* typ. (Plate XIII, figs. 3, 4 & 11).

The following records are of moths reared from larvae in sugar-cane : 284 ♂♂, 304 ♀♀. YARACUY : San Pablo (400 m.) (with *canella*) ; Chivacoa (230 m.), Urachiche (250 m.), Yaritagua (400 m.) (with *canella* and *saccharalis*). LARA : Rio Turbio valley, around Barquisimeto (350–450 m.) (with *canella* and *saccharalis*) ; Duaca (500 m.) ; El Molino, near Quibor (800 m.) ; El Tocuyo valley (600–750 m.), Humocaro Bajo (900 m.) (with *canella*, *impersonatella* and *saccharalis*) ; Sicarigua (475 m.) (with *saccharalis*). TRUJILLO : Campo Elias (1,000 m.) ; Boconó district (1,200–1,500 m.) ; near Carache (575 m.) (with *canella*) ; Trujillo (800 m.), La Concepción (350 m.), Pampanito (350 m.), Motatan (300 m.), Valera (400–600 m.) ; Rio Motatan valley to near Timotes (700–1,500 m.) ; Rio Momboy valley to La Puerta (650–1,700 m.) (with *canella* locally to 960 m.) ; Escuque (1,000 m.) ; San Pedro de Betijoque (750 m.) (with occasional *canella*) ; Betijoque (600 m.). MERIDA : Rio Chama valley, Egido to Mérida (1,100–1,650 m.) (with *saccharalis*) ; Palomitas, El Vigia road (600 m.) (with *saccharalis*). TACHIRA : San Cristobal (850 m.), Santa Ana (700 m.), La Esterlina, Rio Quinimarí (750 m.), Rubio (850 m.) (with *canella* and *saccharalis*) ; Ureña, Rio Táchira, on Venezuela-Colombia frontier (310 m.) (with *saccharalis*). ZULIA : Santa Bárbara, Rio Escalante (sea-level) ; Bobures, S.E. shore of Lake Maracaibo (sea-level), vii.1940 (*C. H. Ballou*).

This is the typical, but not necessarily the parent, form as described from Panama, and as reared by Myers from larvae in sugar-cane at Santa Marta, Colombia (Box, 1935b, p. 329). In Venezuela, this form has not been positively identified from any food-plant other than sugar-cane, in which the larvae have been found from sea-level to 1,700 metres altitude. The most easterly points where f. *busckella* has been found are Chivacoa and San Pablo (Yaracuy), whence it ranges through the western States of Venezuela (see map, fig. 2) and northern Colombia to Panama. If, as seems not altogether improbable, the subsp. *setariae* nov., described below, is the parent form of the species, it is permissible to speculate that f. *busckella* (and f. *falconensis* nov.) may have adopted a domestic habitat confined to cultivated sugar-cane, in which plant it has been spread artificially throughout its range.

The present series of specimens is fairly constant in coloration except for individual variation in the males from light ochreous to shades of brownish as the basic colour ; the dark apical shade, veins and interneural lines in the fore wing are not strongly contrasting. The females are uniformly pale ochreous. Expanse : ♂♂, 21·5–30·0 mm. ; ♀♀, 25·0–37·5 mm.

Forma *falconensis* **nov.** (Plate XIII, figs. 5, 6).

Differs from typical *busckella* in the uniformly dark brownish tint of the fore wing in the male, darker ochreous in the female, and in the strongly contrasting veins and interneural lines, in both sexes ; discocellular and marginal dots conspicuous. In appearance, this form is very similar to *b.* subsp. *setariae* nov., described below, but the male genitalia are as in *b. busckella* f. *busckella* (fig. 1, *c*2). Expanse : ♂♂, 24·0–30·0 mm. ; ♀♀, 30·0–36·5 mm.

13 ♂♂, 8 ♀♀. VENEZUELA, FALCON : Pirítú (200 m.) and Cumarebo (250 m.), reared from larvae in sugar-cane, xi.1949 (*P. Guagliumi*).

The sugar-cane in which f. *falconensis* occurs is grown on a few small farms, of long establishment, situated below the Paraguana peninsula, in the extreme north of the Venezuelan mainland, and is completely isolated from other cane-growing regions of the country (see map, fig. 2).

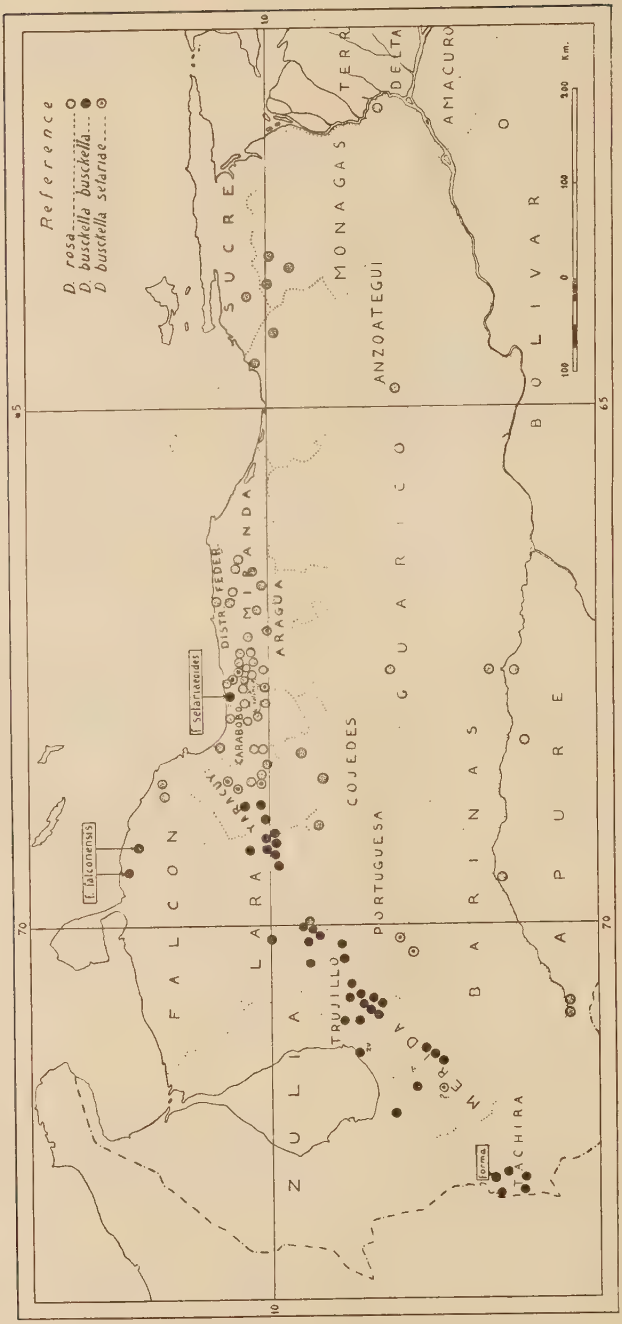
Reference
D. rosa
D. busckella busckella
D. busckella setariae
f. falconensis
f. setariaeoides
Forma
FALCON
LARA
ZULIA
TRUJILLO
MERIDA
TACHIRA
PORTUGUESA
COJEDES
YARACUY
CARABOBO
ARAGUA
MIRANDA
DISTR. FEDER.
SUCRE
MONAGAS
ANZOATEGUI
GUARICO
BARINAS
APURE
BOLIVAR
TERR. DELTA AMACURO
70
65
10
100 0 100 200 Km.

Fig. 2.—Northern Venezuela, showing distribution of *Diatraea rosa* Heinr. and *D. busckella* Dyar & Heinr. It will be observed that whereas *D. b.* subsp. *setariae* nov. occurs at several points within the range of *D. rosa*, there is, with the isolated exception of f. *setariaeoides* nov., no overlapping in the ranges of *D. b. busckella* and *D. rosa*.

Forma *setariaeoides* **nov.** (Plate XIII, figs. 7, 8).

An extremely well-marked moth, in both sexes, differing from other forms of *b. busckella* and also from *b.* subsp. *setariae* nov., in the pronounced dark yellowish (stramineous) tint as the basic colour. The dark apical shade, veins and interneural lines in the fore wing are very strongly contrasting, especially in the ♀, more so than in any other form yet studied ; discocellular and terminal dots large and conspicuous. Expanse : ♂♂, 21·5–27·0 mm. ; ♀♀, 25·5–35·5 mm.

15 ♂♂, 12 ♀♀. VENEZUELA, ARAGUA: Ocumare de la Costa (sea-level), reared from larvae in *Setaria poiretiana* growing by roadside trenches on the edge of a cacao plantation, i, ii and 9.vi.1949, 4.vii.1950 (*H. E. Box*).

An isolated population from outside the geographical range of typical *b. busckella* and f. *falconensis* (see map, fig. 2), apparently confined to a wild food-plant. This form would logically be considered a local race of *b.* subsp. *setariae* nov., were it not that the male genitalia are as in typical *busckella* (fig. 1, *c*2). If *b. setariae* is regarded as the wild parent form of the species, then f. *setariaeoides* could well be the first link in the chain resulting in *b. busckella*. On the other hand, f. *setariaeoides* may simply be an ecological race or strain which has evolved locally.

The grass in which the larva feeds* is widely distributed in north-central and eastern Venezuela, but only at Ocumare de la Costa has it yet been found inhabited by f. *setariaeoides*.

Formae incertae.

At several localities, sometimes where typical *b. busckella* occurs in adjacent fields of sugar-cane, we have reared the species from *Tripsacum* spp., either cultivated or spontaneous nearby. These moths differ from the typical only by their larger size and slightly darker coloration, but insufficient material is available for a complete study.

1 ♀. LARA : Duaca, Hacienda El Milagro (500 m.), reared from larva in *Tripsacum laxum* on sugar-cane plantation, 12.iv.1947 (*H. E. B.*). 1 ♀. LARA : Humocaro Bajo (900 m.), in *T. laxum* close to sugar-cane plantation, 14.xii.1946 (*H. E. B.*). 1 ♀. TRUJILLO : Rio Momboy valley, Mendoza Fria (950 m.), in *T. australe* (with *lineolata*) on roadside embankment in cultivated area, 28.iv.1948 (*H. E. B.*). 2 ♂♂, 2 ♀♀. TACHIRA : near Rinconada, Ureña-Lobatera road (800 m.), in *T. australe* on deforested hillside, 19.iii.1947 (*H. E. B.*).

All of the above are darker in colour than typical *busckella*, and in general larger (expanse of males, 25·5 and 28·0 mm. ; of females, 33·3–38·5 mm.). The series from Táchira rather closely resembles *b.* subsp. *setariae* nov.

Diatraea busckella setariae, subsp. nov. (figs. 1, 2 ; Plate XIII figs. 9, 10, 12).

A well-marked ecological race, conceivably the parent form of the species, with a distinctive character in the male genitalia readily separating it from *D. b. busckella*. It is essentially a " forest insect " and appears to be confined to wild grass hosts of the genus *Setaria*.

♂. Palpus and antenna light buff tinged with cartridge buff. Head light buff at sides, vertex whitish with a darker median line. Thorax cartridge buff tinged with warm buff, slightly darker anteriorly and at base of tegulum. Tergum light buff, proximal two segments clay colour, terminal segments whitish, intermediate segments tinged with fuscous above. Pectus whitish or light buff, tinged with cartridge buff. Legs the same ; posterior tibia with a large conspicuous tuft of long partly fuscous and partly ochreous hair-like scales. Fore wing with the ground colour whitish tinged with light buff giving an ochreous tint to the whole ; the veins and interneural lines strongly contrasting in shades of warm buff and clay colour, darker at base of wing ; a light coloured streak from base of cell extending to margin of wing between veins 5 and 6, enclosing a small fuscous blackish discocellular dot ; a well-defined fuscous shaded area extending diagonally inwards from near apex of wing towards the cell ; a rather broad fuscous shaded streak between veins 4 and 5, extending to below the cell and then merging into the basal shaded area ; a similar fuscous shaded streak along vein 1 c from inner angle of wing to base ; a series of terminal blackish dots between the veins ; marginal scales (cilia) whitish tinged with light buff or cartridge buff. Hind wing cartridge buff tinged with warm buff ; a series of somewhat elongated subterminal fuscous dots between the veins, sometimes obsolete. Underside of fore wing cartridge buff, with the pattern faintly indicated by darker shading ; of hind wing similar to upperside. Expanse : 23·0–30·0 mm.

**Setaria Poiretiana* (Schult.) Kunth (syn. *S. sulcata* Raddi, based on *Panicum sulcatum* Bertol, not *S. sulcata* A. Camus, based on *P. sulcatum* Aubl., which is *S. paniculifera* (Steud.) Fourn.). *S. Poiretiana* is an important weed-grass, called " gamalote ", in Trinidad cacao plantations ; it was erroneously referred to as *S. paniculifera* by the writer (1935a).

♀. Larger ; similar, with the same pattern in the forewing, but the dark shaded areas rather less contrasting than in the ♂, but much more so than in typical *b. busckella* and exactly as in f. *falconensis* ; discocellular dot frequently almost obsolete. Hind wing as in the ♂ but with the terminal dots sometimes more or less obsolete. Expanse : 25·0–37·0 mm.

The frons is moderately prominent, with a sharp point at the apex. The second abdominal segment of the ♂ has lateral retractile processes bearing prominent tufts of hair-like scales. The male genitalia are as in *b. busckella* except for the very distinct shape of the spiny knob-like projection at the base of the harpes (fig. 1, *c* 3). The female genitalia cannot be distinguished from those of *b. busckella* (fig. 1, *d* 2).

Except where otherwise stated, the following records are of specimens reared from larvae in *Setaria paniculifera* :—

101 ♂♂, 92 ♀♀. VENEZUELA. MIRANDA : El Loro valley, near San Casimiro (500 m.), 9.i and 25.ii.1947. ARAGUA : Guayabita, near Turmero (500 m.), also in *Setaria Poiretiana*, various dates 1948–50 ; Maracay-Choroni road (900–1,200 m.), by roadside in montane forest below cloud zone (with occasional *D. pittieri* sp. nov.), 24.iii and 28.vi.1949. CARABOBO : near Yuma promontory, S. of Lake Valencia, between Magdaleno and Guigue (450 m.), by roadside in deciduous forest, also in *Setaria vulpiseta* (rare), various dates 1947–50. YARACUY : Tapia (50 m.), and near Marin (120 m.), by roadside in partially cleared evergreen forest, 30.iii and 28.viii. 1949 ; S. of San Felipe (150 m.), in cultivated area ; Upper Rio Nirgua valley (600 m.), in a few isolated plants growing probably under primitive conditions in a ravine in deciduous forest, 29.iii and 6.v.1949. PORTUGUESA : near Rio Boconó, close to boundary with Barinas State (175 m.), 30.i.1950. BARINAS : 7 kms. E. of Barinas, near Rio Santo Domingo (180 m.), 31.i.1950. ? MERIDA : El Vigia road, 8 kms. N. of Estanques, Rio Chama valley (650 m.), 23.iii.1947 (no adults were reared and the identification remains to be confirmed) (*H. E. Box, P. Guagliumi* and *P. Paredes*).

HOLOTYPE ♂, ALLOTYPE ♀, in British Museum, from the Yuma series. PARATYPES from Yuma, Marin and near San Felipe.

The larvae were entirely absent in large beds of *Setaria paniculifera* growing luxuriantly on the Guasdualito-El Amparo road, Upper Apure, which were very thoroughly examined in February, 1950.

Hybrids between *D. rosa* **and** *D. busckella.**

In the laboratory, numerous attempts have been made to rear hybrids from these two species. Fertile eggs were obtained from the following crosses : (i) *rosa* ♀×*b. busckella* ♂, (ii) *b. busckella* ♀×*rosa* ♂, (iii) *b. busckella* ♀×*b. setariae* ♂, and (iv) *b. setariae* ♀×*rosa* ♂. The larval mortality was very high in all instars, and to date (August, 1950) in only two series of experiments (both *rosa* ♀×*b. busckella* ♂) were adult moths successfully reared, resulting in four small males (expanse 22·5–24·5 mm.) with the characters of *rosa* but much paler in colour than is usual in that species. This investigation proceeds.

Diatraea andina, sp. nov. (fig. 3 ; Plate XIII, figs. 13, 14).

A large, broad winged, dark coloured species, with the pattern of the fore wing suggestive of that of *saccharalis* greatly exaggerated ; the hind wing in both sexes is dark shaded. The genitalia suggest a relationship with *D. fuscella* Schs. (Mexico, Guatemala, Costa Rica).

♂. Palpus warm buff tinged with fuscous at sides and beneath. Antenna cartridge buff. Head and thorax warm buff tinged with some fuscous, especially in front and at base of tegulum. Tergum with proximal two segments clay colour ; apical segment whitish or light buff ; intermediate segments warm buff tinged with fuscous. Pectus

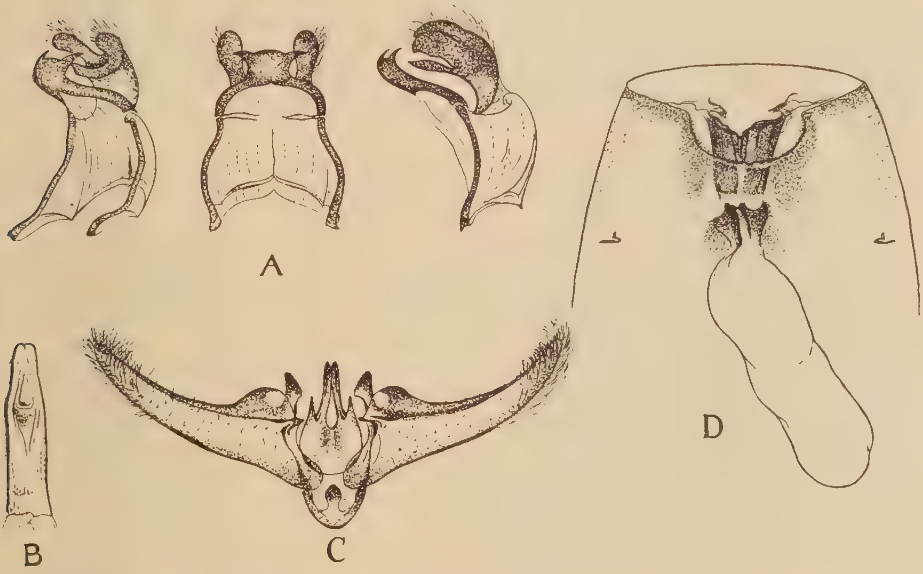

Fig. 3.—*Diatraea andina*, sp. n. Genitalia. (*a*) ♂, Tegumen, uncus and gnathos. (*b*) ♂, Aedeagus. (*c*) ♂, Harpes, vinculum and anellus. (*d*) ♀, Genital opening and bursa copulatrix.

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*From the available evidence, *D. rosa* and the various forms of *D. busckella* may be regarded as together forming a " supraspecies ", in the sense of Huxley (1942, p. 407), *i.e.*, " consisting partly of subspecies, partly of semispecies or full species, all showing geographical (or ecological) replacement ". See also Huxley's interpretation of *Artenkreis* (Rensch) in the same work (p. 179).

No constant characters have been found which enable the larvae and pupae of *rosa* to be distinguished from those of *busckella*, and neither can the larvae of the various forms of the latter be separated from each other. They all, however, exhibit the usual seasonal phases, including the unspotted aestivating form which remains quiescent through most of the dry season.

cartridge buff ; venter the same, apical segment whitish. Legs light buff or cartridge buff ; hind tibia densely clothed for most of its length with moderately long fuscous-tipped scales, not grouped to form a tuft. Fore wing with the ground colour light buff or cartridge buff, accentuated with warm buff or fuscous on the veins and interneural lines ; costal area more uniformly warm buff tinged with fuscous ; an area on the inner margin below vein 1 c from apex of that vein to the base of the wing light buff with numerous scattered individual fuscous-tipped scales ; an elongated light coloured streak surrounding the cell, in the centre of which is a black discocellular dot ; two conspicuous dark diagonal lines, consisting of fuscous scales on and between the veins, the inner one extending from a dark triangular area at apex of wing, downwards and inwards, curving slightly around the cell, and thence straight to base of wing ; the outer one, sometimes resolved into a series of elongate fuscous dots on the veins, in a wide curve from the dark apical shade to vein 1 c at about one-third its length from its apex ; a terminal series of small blackish interneural dots ; marginal scales (cilia) cartridge buff or warm buff tipped with fuscous. Hind wing cartridge buff more or less uniformly shaded with fuscous accentuated subterminally, especially on the veins, the termen shaded with fuscous accentuated interneurally. Underside of fore wing cartridge buff shaded with fuscous except subterminally and on the elongated area surrounding the cell ; the terminal dots sharply defined ; of hind wing similar to upperside. Expanse : 31·0–36·0 mm.

♀. Larger, lighter and more uniformly coloured, with the wing pattern less sharply defined. Fore wing with the general colour cartridge buff, the veins and interneural lines tinged with warm buff or fuscous ; discocellular dot small ; the two diagonal lines less sharply defined, the outer one resolved into a series of elongate fuscous dots. Hind wing and underside of both wings as in the male but lighter and more uniform. Expanse : 35·0–41·5 mm. ; one specimen, excluded from the type series, measures 31·0 mm.

The frons is strongly cone-shaped, with a prominent apical point. The lateral retractile processes on the second abdominal segment of the male bear numerous *short* stiff brownish hair-like scales.

9 ♂♂, 15 ♀♀. VENEZUELA, Andean region. LARA (at boundary with PORTUGUESA) : near Guarico (1,200–1,350 m.), reared from larvae in *Pennisetum bambusiforme* and *P. peruvianum* on steep roadside embankment in secondary woodlands, 4.iv.1947 (*H. E. Box*), 24.iv. and 4.v.1948 (*H. E. B. & P. Guagliumi*). PORTUGUESA : Upper Chabasquen valley, 5–7 kilometres from last locality (1,150–1,250 m.), in *P. bambusiforme*, 24.iv.1948 (*H. E. B. & P. G.*). TRUJILLO : Boconó-Trujillo road, Quebrada San Rafael (1,850 m.), in *P. bambusiforme*, 26.iv.1948 (*H.E.B. & P.G.*). MERIDA : 2 kilometers S. of Mérida (1,600 m.), in *P. peruvianum*, 25.iii.47 and 7.iv.1949 ; Egido-Jajó road at Quebrada La Cruz (1,700 m.), in *P. peruvianum*, 10.iv.1949 (*H.E.B.*). TACHIRA : lower slopes of Páramo de la Negra, near La Grita (1,575 m.), in *P. peruvianum*, 22.iii.1947 ; Upper Rio Torbes, near Cordero (1,200–1,300 m.), in *P. peruvianum*, 17 and 24.iii.1947 (*H.E.B.*).

HOLOTYPE ♂ and ALLOTYPE ♀, from near Cordero ; PARATYPES from other localities named above.

Diatraea pittieri, sp. nov. (fig. 4 ; Plate XIII, figs. 15, 16).

A small but very distinctively marked species (especially in the male), which by the structure of the genitalia in both sexes is closely related to *D. minimifacta* Dyar. There is a greater degree of difference—almost to the point of dimorphism—between the sexes than is usual in the genus, the fore wing of the female being almost uniformly brownish and without the conspicuous pattern of the male.

♂. Palpus cartridge buff tinged with fuscous at sides and below. Antenna setose below, shaft honey-yellow with cartridge buff scales above. Head cartridge buff with a dark median line on vertex. Thorax cartridge buff above, warm buff tinged with fuscous at sides and on base of tegulum. Pectus, legs, tergum and venter whitish tinged with light buff, proximal two segments of tergum ochraceous tawny, apical segments silvery whitish. Fore wing with ground colour whitish on inner margin, but for the most part brownish at base, along costa and the outer margin, the surface sparsely irrorated with scattered individual fuscous scales ; the veins and interneural lines contrasting in shades of warm brown or fuscous ; a minute black discocellular dot, surrounded by an elongate whitish area in the cell ; an ochraceous tawny streak through and extending slightly beyond the cell ; two dark diagonal lines, formed of fuscous scales on and between the veins, the inner one from apex to middle of vein 7 above the cell, thence beyond the cell (merging with the ochraceous tawny streak) in a wide curve to inner margin midway between apex of vein 1 c and base of wing ; the outer one, tending to be resolved into elongate dots on and between the veins, from same point below apex, curving more or less parallel to the inner line, and for the most part on a whitish ground colour, to inner margin at base of vein 1 c ; a terminal series of conspicuous black interneural dots each partially surrounded by white scales ; cilia in shaded lines of wood brown or fuscous scales. Hind wing whitish tinged with light buff ; a terminal series of five interneural fuscous dots ; cilia light buff. Underside of fore wing warm buff tinged with fuscous, with the pattern of the upperside faintly indicated. Expanse : 18·0–21·5 mm.

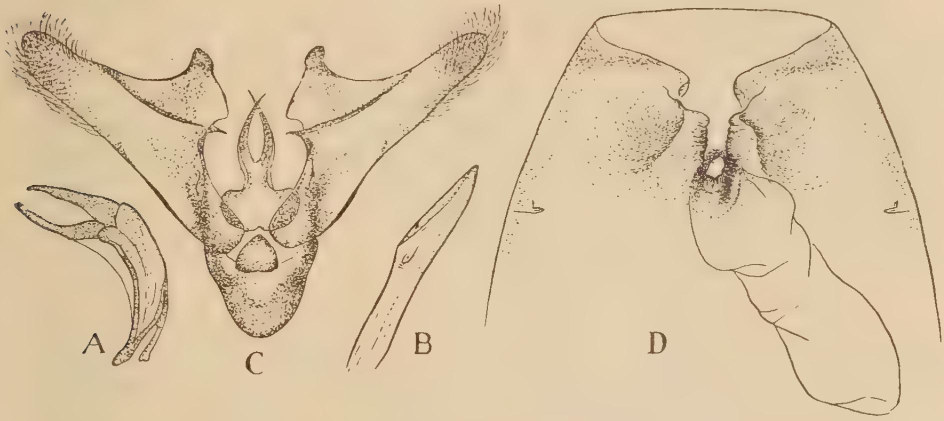

Fig. 4.—*Diatraea pittieri*, sp. n. Genitalia. (*a*) ♂, Tegumen, uncus and gnathos ; (*b*) ♂, Aedeagus. (*c*) ♂, Harpes, vinculum and anellus. (*d*) ♀, Genital opening and bursa copulatrix.

♀. Larger ; darker ; the fore wing almost uniformly warm brown tinged with cinnamon, without any whitish in the ground colour ; the pattern of the male is faintly indicated ; discocellular and terminal dots as in the male. Hind wing whitish tinged with cartridge buff marginally, terminal dots almost obsolete. Expanse : 20·0–26·5 mm.

The frons is gently rounded, not protuberant.

10 ♂♂, 9 ♀♀. Venezuela, Aragua : Aragua National Park: Rancho Grande, Portachuelo Pass (1,100 m.) and nearby (900–1,000 m.), reared from larvae in *Setaria paniculifera* and (rarely) in *Paspalum saccharoides*, on roadside embankments in montane cloud-forest, 29.xii.1948, 4.i and 27.x.1949, 4.vii.1950 and in *Paspalum virgatum* in secondary forest zone (600–750 m.), 30.viii.50 ; near summit of Maracay-Choroní road (900–1,200 m.), in *S. paniculifera* (with *b. setariae*) by roadside in

montane cloud-forest, 24.iii.1949 (*H. E. Box*) ; Guayabita, near Turmero (500 m.), in *Setaria Poiretiana* (with *b. setariae*), 14.vii.50 (*P. Paredes*).

HOLOTYPE ♂ and ALLOTYPE ♀ from Rancho Grande ; PARATYPES from Rancho Grande and Maracay-Choroní road.

Dedicated to the memory of the late Dr. Henri Pittier, Venezuela's foremost naturalist for many decades, to whose initiative and perseverance we are indebted for the preservation of the Aragua National Park and the establishment at Rancho Grande of a station for biological research.*

Montane cloud-forest is a new and most unexpected habitat in which to find a species of *Diatraea.*

Diatraea silvicola, sp. nov. (fig. 5 ; Plate XIII, figs. 17, 18).

A small light-coloured species related to *D. savannarum* Box (British Guiana, Venezuela), but without the ochraceous tawny scales present in the fore wing of that moth, and differing in other details of the wing pattern. The genitalia of both sexes are characteristic, but attention is drawn especially to the shape of the anellus in the male and the serrate-striate area in the bursa of the female. The male anellus is of similar form to, but is smaller than, that of *D. lentistrialis* Hamps. (Argentina) which belongs to the same section of the genus.

♂. Palpus cartridge buff tinged with some fuscous at sides and below. Antenna setose below, shaft honey yellow with sparse light buff scales above. Head whitish or cartridge buff, with a narrow dark streak on vertex. Thorax cartridge buff tinged with some fuscous at sides and at base of tegulum. Tergum, pectus, legs and venter whitish to light buff, proximal two segments of tergum cinnamon. Fore wing with ground colour whitish or light buff, covered with brownish scales at base and along the costa ; the veins lined with similar scales, interneural lines warm buff, not very strongly contrasting ; a small black discocellular dot ; two diagonal lines formed of dark-coloured scales on and between the veins, the inner one almost straight

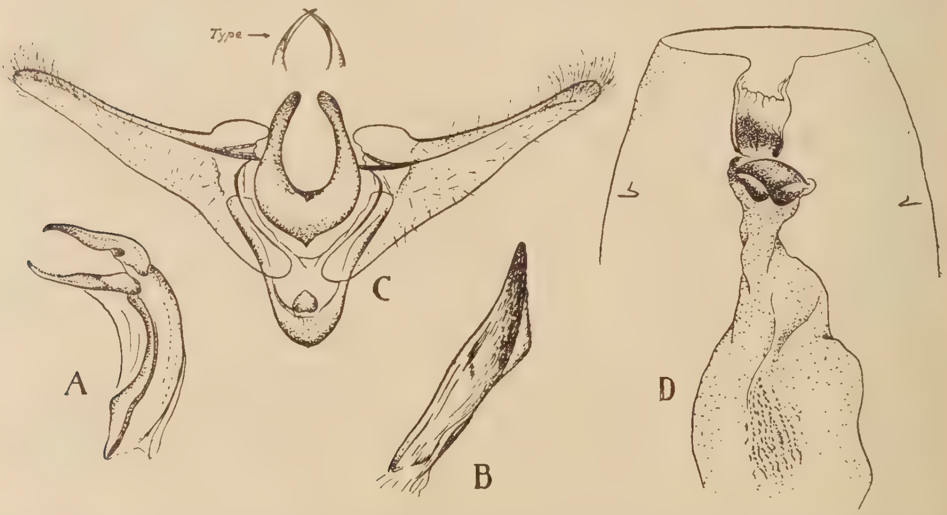

Fig. 5.—*Diatraea silvicola*, sp. n. Genitalia. (*a*) ♂, Tegumen, uncus and gnathos ; (*b*) ♂, Aedeagus. (*c*) ♂, Harpes, vinculum and anellus. (*d*) ♀, Genital opening and bursa copulatrix.

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*Beebe and Crane (1947) have given a general description of this region. The type locality of *D. pittieri* sp. n. is shown on their map (text-fig. 4) and is just below the point where the photograph (Pl. I, fig. 1) was taken.

from apex of wing to below apex of cell, thence bent slightly inwards to join inner margin halfway between apex of vein 1 c and base of wing ; the outer one, resolved into elongate dots on and between the veins, from near apex of vein 8 in a gentle curve inwards to inner margin at apex of vein 1 c ; a terminal series of conspicuous black interneural dots ; cilia with parti-coloured scales forming alternating rows of dark and light shades of brownish. Hind wing light buff tinged with some warm buff at apex and on a narrow sub-marginal band ; cilia whitish. Underside of fore wing cartridge buff with the pattern of the upperside faintly indicated. Expanse : type 17·0 mm., paratype 13·0 mm.

♀. Similar ; larger ; colour and pattern as in the male but slightly darker ; fore wing slightly narrower. Expanse : 20·5 mm.

The frons is rounded and slightly protuberant. In the specimens examined, vein 10 in the fore wing is free from the cell. The second abdominal segment of the male has lateral retractile processes bearing small tufts of hair-like scales.

HOLOTYPE ♂, ALLOTYPE ♀, and 1 PARATYPE ♂. VENEZUELA.—APURE : Guasdualito-El Amparo road, Upper Apure (180 m.), reared from larvae in *Setaria vulpiseta* (with *D. brunnescens*) growing under trees on edge of forest, 5.ii.1950 (*H. E. Box & P. Guagliumi*).

Summary.

Records are given of the localities (with altitudes) and habitats of 11 species of *Diatraea* Guild., all reared from known food-plants in northern Venezuela. The larvae of several of these moths are important pests of sugar-cane and maize. Among the eight previously described forms, *D. busckella rosa* Heinr. is here raised to specific rank, and *D. busckella* Dyar & Heinr. is treated as a polytypic species, in which typical subsp. *busckella* has three well-defined forms, two of them, *viz.*, f. *falconensis* nov. and f. *setariaeoides* nov., being described, while others are indicated ; the subsp. *setariae* nov. is also described. The diagnostic characters of *D. rosa* and *D. busckella* are discussed and their genitalia are illustrated. The various subspecies and forms of *D. busckella* are discussed in relation to each other and to *D. rosa*, these two species being considered as together comprising a supraspecies. Hybrids have been reared in the laboratory among certain of the components.

Three new species, *D. andina*, *D. pittieri* and *D. silvicola*, are described and illustrated, all from northern Venezuela.

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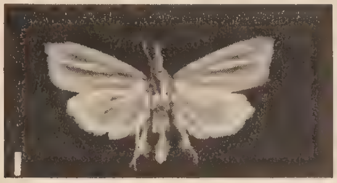
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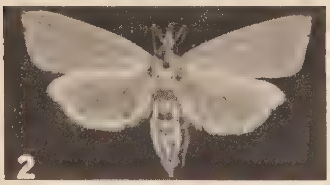
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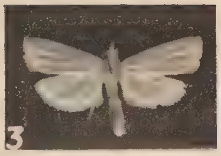
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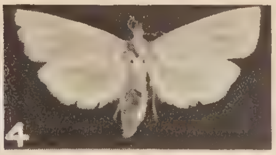
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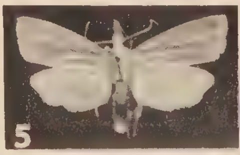
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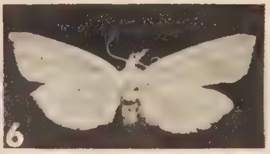
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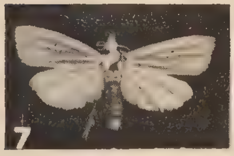
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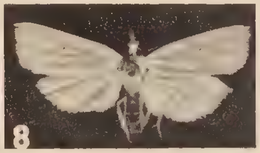
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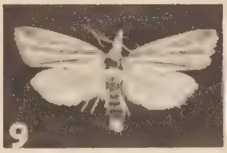
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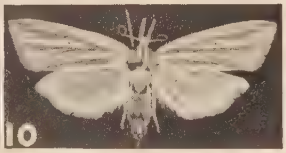
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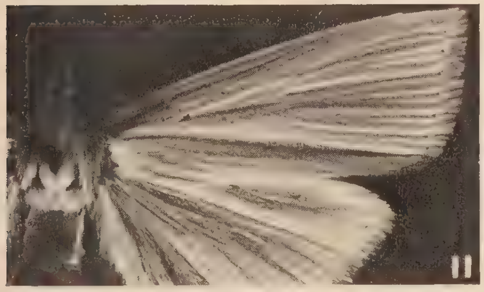
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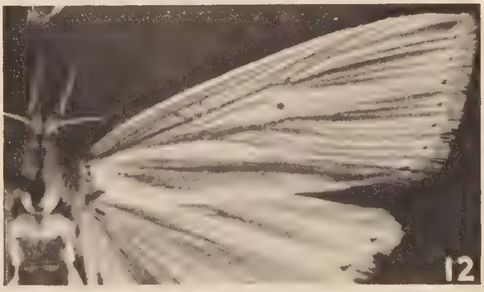
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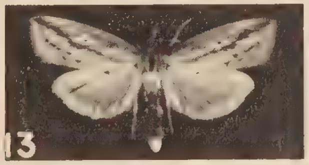
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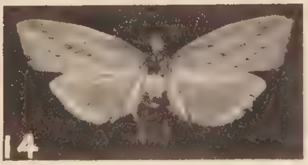
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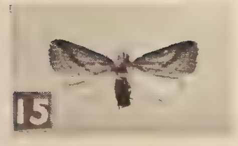
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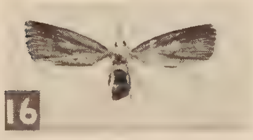
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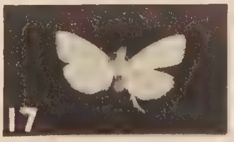
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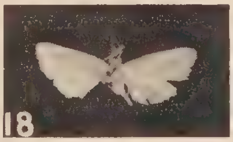
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Venezuelan *Diatraea.*

FIGS. 1, 2. *D. rosa* Heinr. FIGS. 3, 4. *D. busckella busckella* Dyar & Heinr., forma typica. FIGS. 5, 6. *D. busckella busckella* Dyar & Heinr., forma *falconensis* nov. FIGS. 7, 8. *D. busckella busckella* Dyar & Heinr., forma *setariaeoides* nov. FIGS. 9, 10. *D. busckella* subsp. *setariae* nov. FIG. 11. *D. busckella* Dyar & Heinr., forma typica ♂ (x 4). FIG. 12. *D. busckella* subsp. *setariae* nov. ♂ (x 4). FIGS. 13, 14. *D. andina* sp. nov. FIGS. 15, 16. *D. pittieri* sp. nov. FIGS. 17, 18. *D. silvicola* sp. nov.

SPECIFIC SUSCEPTIBILITY TO HCN AND THE AMOUNT OF HCN RECOVERED FROM FUMIGATED INSECTS.

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Various species of insects differ greatly in their specific resistance to the same insecticide. To cite an extreme case, it has been shown experimentally by Carpenter and Moore (1938) that a concentration of approximately 0·5 mg. per litre hydrogen cyanide kills 99 per cent. of the flour beetles, *Tribolium confusum* Duv., in a 10 minutes' exposure period, whereas a concentration of about 270 mg. per litre is required to kill 99 per cent. of the rice weevils, *Sitophilus oryzae* (L.), under the same conditions. This means a difference in dosage of about 500 times. Even within an insect species, a new resistant strain may develop as a result of constant application of the same insecticide. Attention to such a phenomenon was first drawn by Melander (1914) who observed strains of San José scale resistant to lime sulphur sprays. Subsequently, a strain of the California red scale of citrus, *Aonidiella aurantii* (Mask.), was noticed to be resistant to HCN fumigation in 1914 and that of *Saissetia oleae* (Bern.) in 1915 (Quayle, 1938). Recently Lindquist and Wilson (1948), and Barber and Schmitt (1948, 1949) in U.S.A., and Keiding and van Deurs (1949) in Denmark have shown experimentally the development of resistance to DDT in house-flies. Thus the question of resistance of insects to insecticides has an important bearing on their use.

The difference in the resistance of insects to a fumigant may be due to a bewildering complex of factors. For a systematic analysis of these factors, the determination of the amount of poisonous gas actually taken in and retained by the insect is very important. Carpenter and Moore (1938) were the first to study this intake or sorption of HCN by a few species of insects using a manometric method. Lindgren and Sinclair (1944, 1945) estimated the sorption of HCN by recovering the amount of HCN sorbed by the insect, and estimating this amount colorimetrically. These investigators found that sorption of a fumigant by insects is correlated with their specific susceptibility. The present contribution partially corroborates the above finding and attempts, further, to analyse " Total Effective Resistance " into two components, *viz.*, " Surface Resistance " and " Internal Resistance ".

Material and Apparatus.

The insects used in these investigations were (1) adults of *Tribolium castaneum* (Hbst.) (Col., Tenebrionidae), (2) the seventh-stage caterpillars of *Corcyra cephalonica* (Staint.) (Lep., Pyralidae), (3) first- and second-instar nymphs of *Drosicha* sp. (Hom., Coccidae), (4) third- and fourth-instar nymphs of *Drosicha* sp. and (5) the adult females of *Drosicha* sp. *T. castaneum* and *C. cephalonica* were reared on crushed jowar (*Andropogon sorghum*) seeds in a room with the temperature controlled between 80° and 85°F. and the humidity between 80 per cent. and 95 per cent. The adult females of *Drosicha* sp. were collected from *Grevillea robusta* (Proteaceae) and the nymphs from *Prosopis juliflora* (Mimosaceae). Samples were taken at random in order to minimise the extent of variation in age and sex. All the insects were preconditioned by keeping them without food in a controlled temperature of 80° to 84°F. and in a humidity of 35 to 50 per cent. for 24 hours.

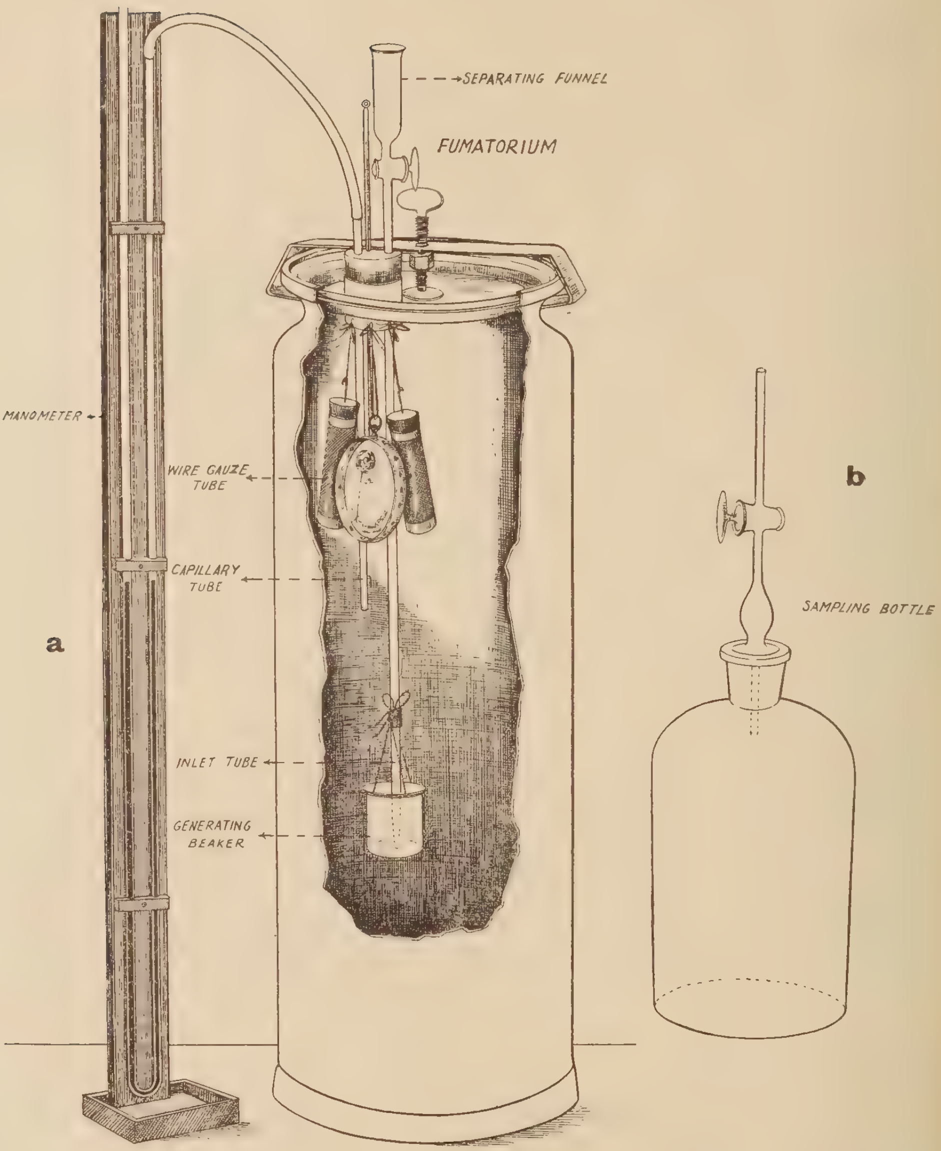

SEPARATING FUNNEL
FUMATORIUM
MANOMETER
WIRE GAUZE TUBE
CAPILLARY TUBE
a
INLET TUBE
GENERATING BEAKER
b
SAMPLING BOTTLE

Fig. 1.

The apparatus used in the investigation consisted of a *fumatorium* (fig. 1 *a*), *sampling* bottles (fig. 1 *b*) a *microstill i.e.*, the apparatus for microdistillation (fig. 2), *sorption apparatus* (fig. 3), *reaction containers*, which were ordinary pyrex test tubes of $\frac{3}{4} \times 6$ in. size, graduated at 10 ml. and 25 ml., *constant humidity desiccators* and a *lumetron*, model 400–A (Photo volt Corporation, New York City), using a blue green filter (4,900 Ångström units).

The chemicals and reagents used during the course of investigations were :—

1. Picric acid solution ... ... (5 gm. in 500 ml. solution).
2. Sodium carbonate solution ... ... (25 gm. in 500 ml. solution).
3. 10 N sulphuric acid.
4. Sodium hydroxide solution ... ... (10 gm. in 500 ml. solution).
5. Potassium iodide solution ... ... (10 gm. in 500 ml. solution).
6. Silver nitrate N /100.
7. Sodium cyanide solution N /100, N /1000, N /10,000.

Technique.

The main operations carried out during the investigations were : (i) fumigation, (ii) examination of the fumigated insects and (iii) recovery of HCN from the insects immediately after fumigation. The techniques used are essentially those of Lindgren and Sinclair (1944) and Sinclair and Ramsey (1944) but are being described here as some variations had to be made in the details of the working and fumigation apparatus.

Fumigation.

The fumatorium consisted of a single metal cylinder in which the insects to be fumigated were enclosed in wire-gauze tubes hung from the lid (fig. 1 *a*). A partial vacuum, equivalent to about 48 ins. of water, was created within the fumatorium with the help of a filter pump, and hydrogen cyanide was generated by dropping a known volume of diluted sulphuric acid from the separating funnel, through the inlet tube, on to the sodium cyanide kept in the generating beaker. The ingredients were used in the ratio of 1 gm. of sodium cyanide, 2·5 ml. of water and 1·0 ml. of concentrated sulphuric acid. The completion of the reaction between sulphuric acid and sodium cyanide was indicated when the manometer registered a steady pressure. The remaining vacuum was dissipated and the pressure brought to that of the atmosphere by letting in air ; this operation also ensured uniform distribution of the HCN. The exposure period in all the experiments was kept at 30 minutes. During fumigation two samples of the air-HCN mixture were taken out in evacuated sampling bottles (fig. 1*a* in which the tube carrying the separating funnel was also used for partial evacuation of the fumatorium and for taking out the samples of HCN-air mixture), containing 5 or 10 ml. (according to the concentration of HCN to be sampled) of 2 per cent. sodium hydroxide which absorbed all the HCN in the bottle in about half an hour. This dissolved HCN was titrated with standardised N /100 silver nitrate solution by the modified Leibig method as given by Cupples (1933), using 4 ml. of 2 per cent. potassium iodide as indicator.

An effort was made to keep the pre-fumigation and post-fumigation temperatures of the insects under treatment within a narrow range (80°-86°F.) in all the experiments. Pre-fumigation and post-fumigation humidities were also kept between 35 and 50 per cent. in all the experiments. Humidity during the fumigation was not controlled but was recorded at the end of the treatment. The exact temperature and humidity conditions during each experiment were recorded.

Examination of fumigated insects.

Six batches of each species of insect, in separate wire-gauze tubes, were fumigated together at each concentration of HCN, three batches being used for recovery of HCN and the other three for mortality studies as three replicates. Three replicates of control insects also were maintained for each experiment. Mortality counts were taken 48 hours after fumigation. The number of insects used per replication for the purpose of mortality counts was usually 100 *T. castaneum* adults, 10 *C. cephalonica* caterpillars, 10 adult females of *Drosicha* sp. and 30 nymphs of *Drosicha* sp. Specimens which showed any movement of the body at the end of the 48 hours after fumigation were regarded as alive and the remainder were examined on a warm metal plate (temp. 119°F.). Those insects which showed movement of the legs whilst on a warm plate were also taken to be alive and the others were regarded as dead. Percentage mortality was adjusted according to Abbott's formula (1925).

Recovery of HCN from fumigated insects.

HCN was recovered separately from each of the three batches of insects fumigated at each concentration. The alkaline picrate method, as standardized by Sinclair and Ramsey (1944), was used. Before starting the fumigation, the insect samples destined for the recovery of HCN were counted and weighed on a chemical balance (correct to 0·1 mg.). Immediately after fumigation each insect sample was carefully transferred to a distilling flask (fig. 2) and about 1·5 ml. of distilled water added in order to submerge the insects completely. The receiving tube of the microstill contained 1 ml. of 5 per cent. solution of sodium carbonate. The distilling flask was stoppered tightly and connected to the receiving tube which was then connected to the filter pump, adjusted to give a slow but steady flow of bubbles through the sodium carbonate solution (rate about 1–2 bubbles per second). The receiving tube was kept immersed in an ice bath. A drop of 10 N sulphuric acid was then added to the distilling flask through the aeration tube and the distilling flask placed in boiling water. Distillation was continued for 20 minutes.

After distillation, the contents of the receiving tube were transferred to a reaction tube. The receiver and the delivery tube of the microstill were carefully washed at least three times with distilled water and the wash water added to the distillate, taking care that the volume in the reaction tube did not exceed 9 ml. One ml. of picric acid solution was added to the distillate and the volume brought to 10 ml. The reaction tube was heated in boiling water for exactly three minutes and then cooled and kept

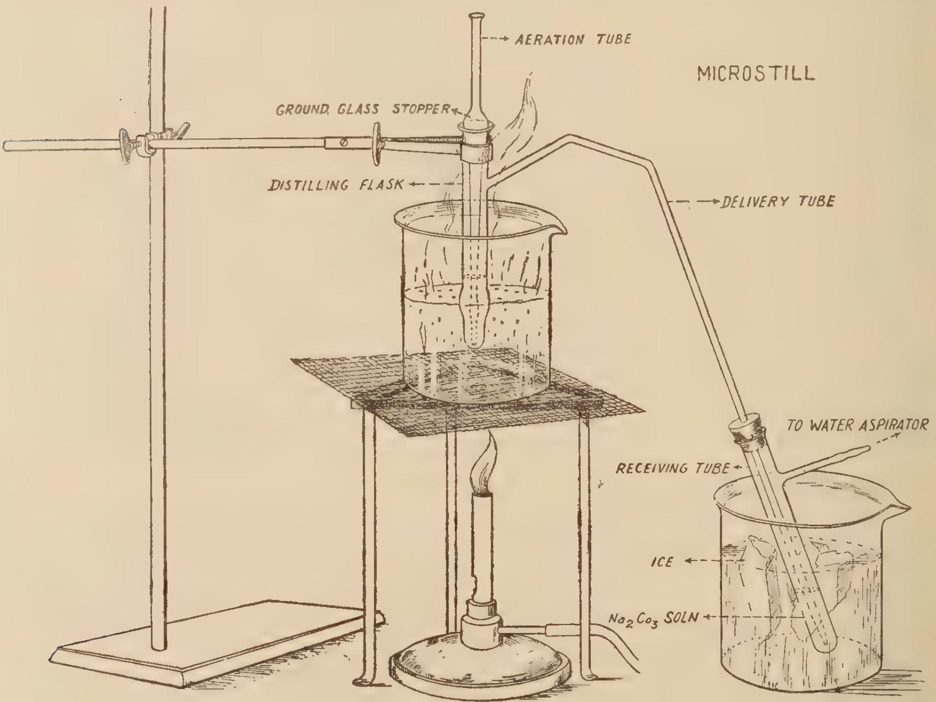


Fig. 2.

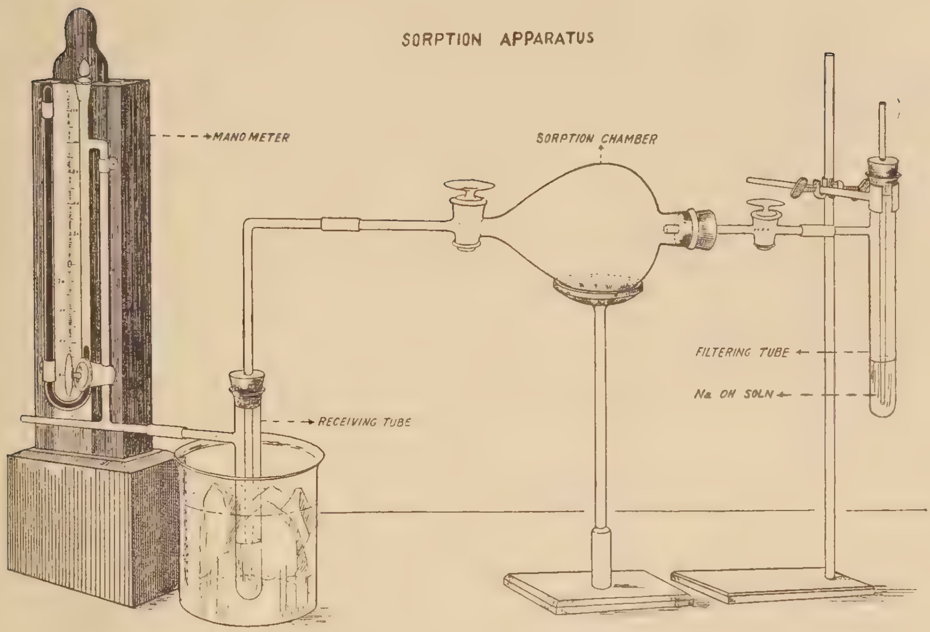

SORPTION APPARATUS
MANOMETER
SORPTION CHAMBER
FILTERING TUBE
Na OH SOLN
RECEIVING TUBE

Fig. 3.

in a water bath at 91°–93°F. for 20 hours. Thereafter, the contents were diluted to 25 ml. with distilled water and thoroughly mixed. A blood red colour develops in the reaction tube ; the intensity of this colour was determined with the help of the lumetron, using a blue green filter of 4,900 Ångström units. In each experiment, a blank solution was prepared by mixing 1 ml. of the picric acid and 1 ml. of the sodium carbonate solution and treating the product exactly in the same manner as the distillate. While taking the lumetron readings, the reading of the blank solution was always kept at 100. From the readings obtained the amount of HCN recovered was calculated by means of a calibration curve (fig. 4). This curve was prepared by

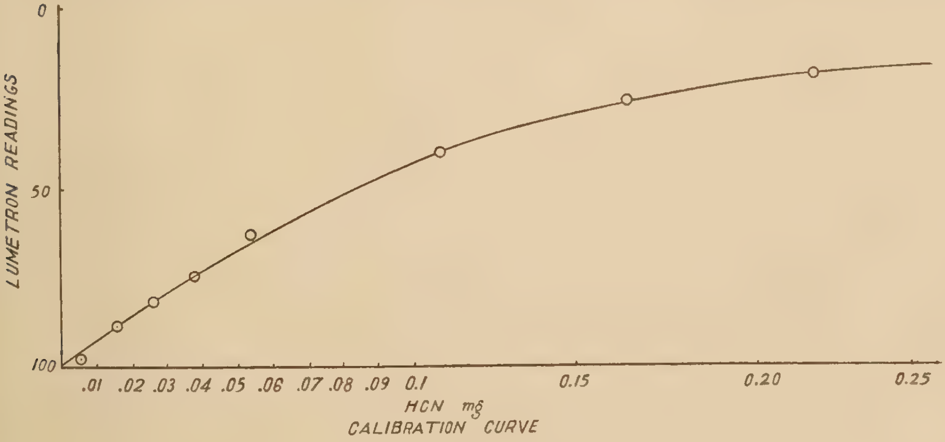


Fig. 4.

taking different known quantities of the standardised sodium cyanide solutions of N/1,000 and N/10,000 strength and treating these in the same manner (for the development of colour) as the distillates described above. The readings of the lumetron obtained for the known quantities of HCN were then plotted on the graph and the curve obtained is shown in fig. 4. The calibration curve was again prepared after about 3 weeks to see if there were any alteration.

Preliminary Experiments.

Average weight of insects.

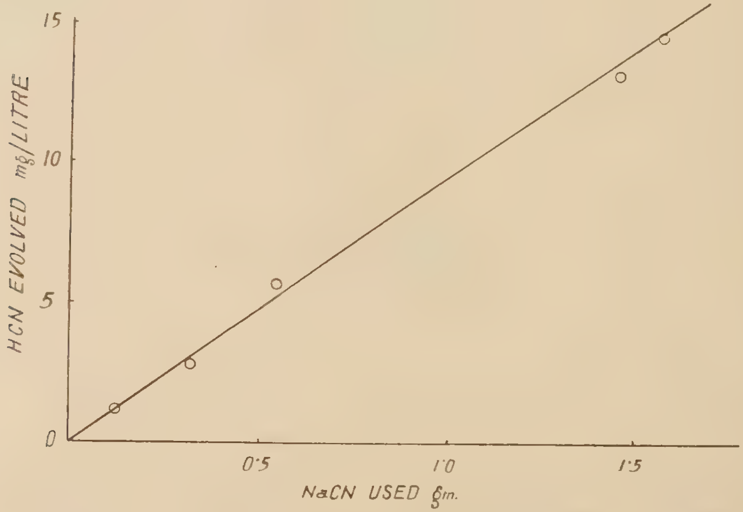
The average weights of the insects determined during the course of the investigation are given in Table I.

TABLE I.

Average weights of insects.

Insect	Stage of development	No. of insects weighed	Average weight of insect in mg.
T. castaneum (Hbst.)	Adults	4,946	1·4
Rhizopertha dominica (F.)* ...	Adults	500	1·056
C. cephalonica (Staint.)	7th stage caterpillars	533	15·84
Drosicha sp.	Nymphs, 1st and 2nd stage ...	1,042	0·5878
Drosicha sp.	Nymphs, 3rd and 4th stage ...	525	3·215
Drosicha sp.	Adult females	48	181·3

*First included in the experiments but was ultimately omitted owing to lack of material.



RELATIONSHIP BETWEEN Na CN USED AND HCN EVOLVED DURING FUMIGATION IN FUMIGATION CYLINDER 42·2 LITRE CAPACITY

Fig. 5.

Amount of sodium cyanide used and the concentration of HCN gas obtained in the fumatorium.

Five experiments were carried out in order to get an approximate idea of the amount of sodium cyanide needed to produce the required concentration of HCN in a fumatorium of 42·2 litres capacity and also the variation in the concentration of HCN in the fumatorium for a period of half an hour. The results are given in fig. 5.

It was found that the concentration of HCN produced by 1 gm. of NaCN varied from 9·1 mg. per litre to 10·2 mg. per litre on different dates. Hence it was decided to determine the actual HCN concentration in each experiment. It was observed that the HCN concentration varied only slightly during the period of 30 minutes.

Effects of distillation and redistillation on the recovery of HCN.

It was necessary to ascertain whether the processes of distillation and redistillation (*vide infra*) affect the recovery of HCN in any way. Known amounts of a standardised sodium cyanide solution were therefore subjected to the same processes of distillation and redistillation as the insects and the amounts of HCN recovered were determined. The results are presented in Table II.

TABLE II.

Effects of distillation and redistillation on the recovery of HCN.

HCN added* (mg.)	Distillation	Redistillation	Lumetron readings
0·0 (blank)	—	—	100·0
0·017 (control)	—	—	89·2
0·017	+	—	89·0
0·017	+	—	88·8
0·017	+	—	89·0
0·017	+	+	89·0

N.B.—(+) means that the operation was performed and (—) means that the operation was not performed.

*Equivalent to the amount of NaCN taken.

The term "blank" means distilled water to which the usual reagents, picric acid and sodium carbonate, were added as in other cases; the "control" contains, besides these reagents, a known amount of standardised sodium cyanide solution. Neither of these were distilled or redistilled. It will be seen that the lumetron readings both in cases of distillation and redistillation were practically the same as that in the control showing that there was no effect of distillation or redistillation on the recovery of HCN.

Effect of volatile substances in the distillates on recovery of HCN.

The chief drawback of the alkaline picrate method of determining microquantities of HCN is that it is not specific. If the process of distillation happens to produce some reducing substance which can be carried over to the distillate that substance may also react with alkaline picrate and give faulty results. Sinclair and Ramsey (1944) reported that *T. confusum* did actually give out such a substance but they could achieve "partial success" in eliminating these impurities by redistilling the distillate at a lower temperature. Hence it was necessary to conduct trials on each of the insects in order to see (a) if such substances were produced, (b) if the result obtained for recovery of HCN was affected by these substances, if present, and (c) if these substances could be eliminated by redistillation. Known amounts of standardised NaCN solutions were added to the insects and the amounts of HCN recovered after distillation and after redistillation were estimated. The results are given in Table III.

TABLE III.

Presence of impurities in the distillate and their effect on the estimation of HCN.

Name and stage of insect	Number used	HCN added* (mg.)	Distillation	Redistillation	Lumetron readings
T. castaneum, adults ...	Nil (blank)	Nil	—	—	100
	Nil (control)	0·017	—	—	89
	50	Nil	+	—	80
	50	0·017	+	+	89
	50	Nil	+	+	100
C. cephalonica, 7th stage caterpillars	Nil (blank)	Nil	—	—	100
	Nil (control)	0·05	—	—	68·5
	10	Nil	+	—	100
	10	0·05	+	—	68·5
Drosicha sp., 1st and 2nd instar nymphs	Nil (blank)	Nil	—	—	100
	Nil (control)	0·0054	—	—	97
	20	Nil	+	—	100
	80	0·0054	+	—	97·6
Drosicha sp., 3rd and 4th instar nymphs	Nil (blank)	Nil	—	—	100
	Nil (control)	0·0054	—	—	97
	50	Nil	+	—	100
	60	0·0054	+	—	97
Drosicha sp., adult females	Nil (blank)	Nil	—	—	100
	Nil (control)	0·0054	—	—	97
	4	Nil	+	—	100
	4	0·0054	+	—	97

*Equivalent to the amount of NaCN taken.

From Table III it will be found that blanks always gave a lumetron reading of 100 and the greater the amount of reducing substances in the distillate the lower the reading of the lumetron. With this criterion it will be found that distillates from unfumigated insects alone gave lumetron readings of 100 in all the species except *T. castaneum* adults in which case it was 80, and these insects were also observed to give off reddish vapours.* It was clear that the distillate from *T. castaneum* adults contained impurities whereas those from other insects did not. Therefore the distillate of *T. castaneum* adults was redistilled in order to get rid of this impurity if possible. The reading after redistillation (89 in the case of insects plus HCN and 100 in the case of insects alone) shows that the impurity could be got rid of. It was not necessary to redistil the distillate of other species as they did not indicate the presence of any impurity.

Main Experiments.

Percentage mortality and HCN recovery.

Observations on (a) percentage mortality and (b) recovery of HCN were made on parallel batches of insects fumigated together with each concentration of HCN. The data are given in Table IV. The relationship between mortality and concentration of HCN is graphed in fig. 6, and that between recovery of HCN and concentration in fig. 7.

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*Alexander and Barton (1943) have studied the properties of this substance and identified it as p-ethylbenzoquinone.—Biochem. J., **37**, p. 463.

TABLE IV.

Percentage mortality in and recovery of HCN from insects exposed to different concentrations of HCN for a period of 30 minutes.

Insect	Stage	Fumigation at :					Number of insects/ replicates	Percentage mortality					Recovery of HCN (mg/100 gm. of insects)			
				Conc. of HCN mg/litre				In control average three replicates	In fumigated insects							
		Temp. °F.	Humidity per cent.	At beginning	At end	Average			Repl. I	Repl. II	Repl. III	Average	Repl. I	Repl. II	Repl. III	Average
T. castaneum ...	Adults	84–85	60	0·87	0·87	0·87	100	2·0	7·9	5·3	3·5	5·6	—	2·2	—	2·2
		85–87	80	1·02	1·04	1·03	100	2·0	51·5	—	16·1	33·8	3·9	6·9	1·2	4·0
		86	70	1·1	1·2	1·15	100	1·0	59	68	48	55·8	3·6	5·2	4·4	4·4
		85	60	2·2	2·15	2·2	100	2·0	88	85	94	89	5·4	6·2	5·3	5·6
C. cephalonica ...	7th stage caterpillars	86–88	60	0·55	0·52	0·54	10	0	0	0	0	0	—	2·7	—	2·7
		84–85	60	0·87	0·87	0·87	10	0	40	40	40	40	9·6	3·9	3·3	5·6
		85–87	80	1·02	1·04	1·03	10	3·3	60	40	44	48	4·5	5·4	4·5	4·7
		86	70	1·1	1·2	1·15	19	0	70	67	—	69	—	6·6	—	6·6
		85	60	2·2	2·15	2·2	10	0	100	90	80	90	13·7	11·3	9·5	11·5
Drosicha sp. ...	1st and 2nd instar nymphs	86–88	60	0·55	0·52	0·54	75	25	—	73	—	73*	—	2·4	—	2·4
		85	90	0·56	0·52	0·54	30	47	50	17	57	41*	—	—	—	—
		85	—	0·75	0·70	0·73	—	—	—	—	—	—	0·2	5·4	—	3·8
		87–88	—	1·04	1·02	1·03	30	8	96	83	90	90	—	—	—	—
		86	70	1·1	1·2	1·15	120	12	—	100	—	100	3·7	—	—	3·7
Drosicha sp. ...	3rd and 4th instar nymphs	86–88	60	0·55	0·52	0·54	30	8·0	13	33	64	37	—	1·4	—	1·4
		85	90	0·56	0·52	0·54	30	24·0	0	34	0	11	—	—	—	—
		85	—	0·75	0·70	0·73	—	—	—	—	—	—	3·5	0·2	1·3	1·7
		87–88	—	1·04	1·02	1·03	30	0·0	—	45	—	45	—	—	—	—
		86	70	1·1	1·2	1·15	122	2·5	94	—	97	96	3·4	3·5	—	3·5
Drosicha sp. ...	Adult females ...	85	90	0·55	0·52	0·54	10	0	0	0	0	0	0·73	0·91	0·86	0·83
		85	82	1·8	1·4	1·6	10	0	0	0	0	0	0·37	0·47	0·0	0·29
		85	95	3·2	3·1	3·15	10	0	55	40	11	35	0·5	1·0	0·0	0·5
		85–86	95	3·7	3·6	3·65	10	0	70	80	90	80	1·3	0·7	1·0	1·0

*The difference in mortality may be due to difference in relative humidity during fumigation.

Pre- and post-fumigation temperature was 80–84°F. and relative humidity 35–50 per cent.

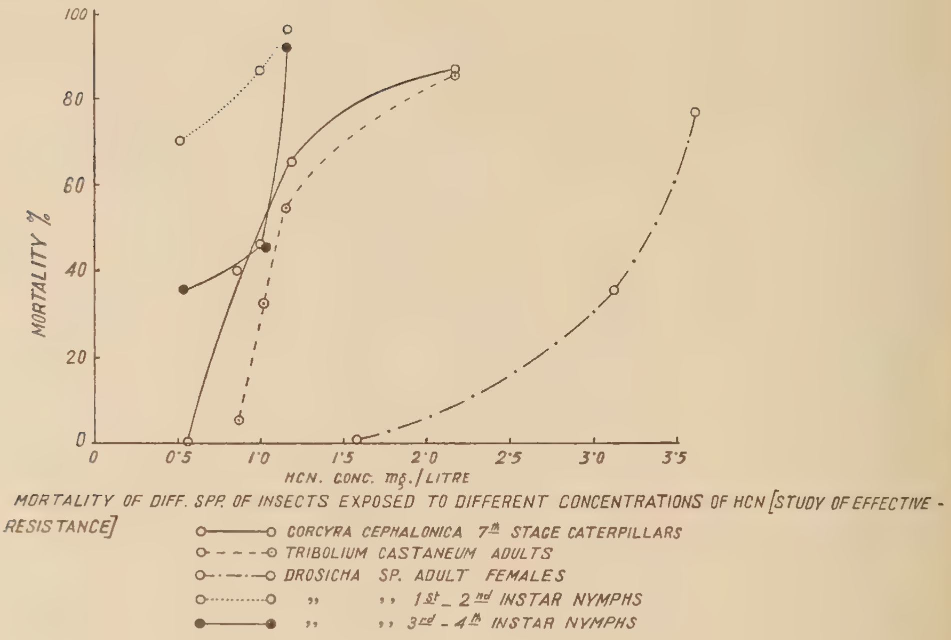

100
80
60
40
20
0
MORTALITY %
0
0'5
1'0
1'5
2'0
2'5
3'0
3'5
HCN. CONC. mg./LITRE
MORTALITY OF DIFF. SPP. OF INSECTS EXPOSED TO DIFFERENT CONCENTRATIONS OF HCN [STUDY OF EFFECTIVE - RESISTANCE]
CORCYRA CEPHALONICA 7th STAGE CATERPILLARS
TRIBOLIUM CASTANEUM ADULTS
DROSICHA SP. ADULT FEMALES
" " 1st _ 2nd INSTAR NYMPHS
" " 3rd - 4th INSTAR NYMPHS

Fig. 6.

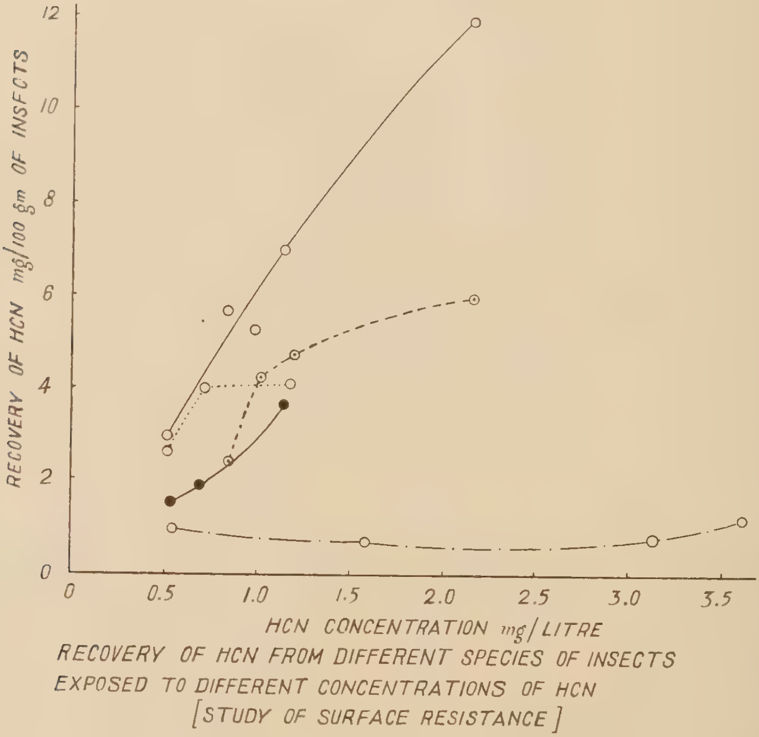
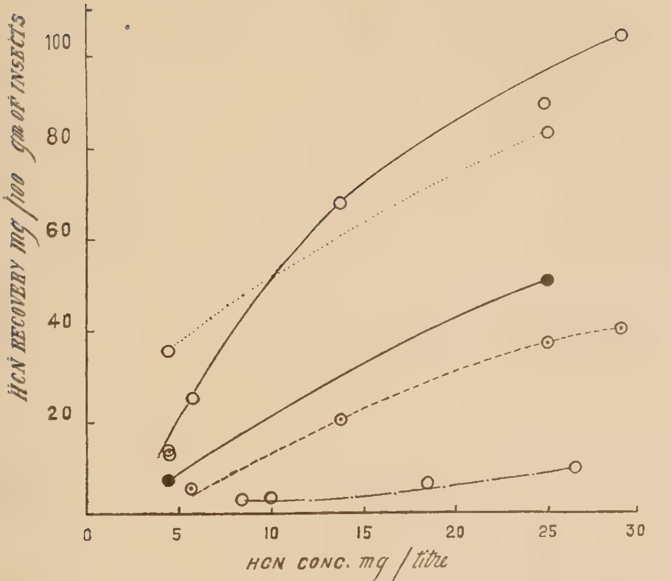
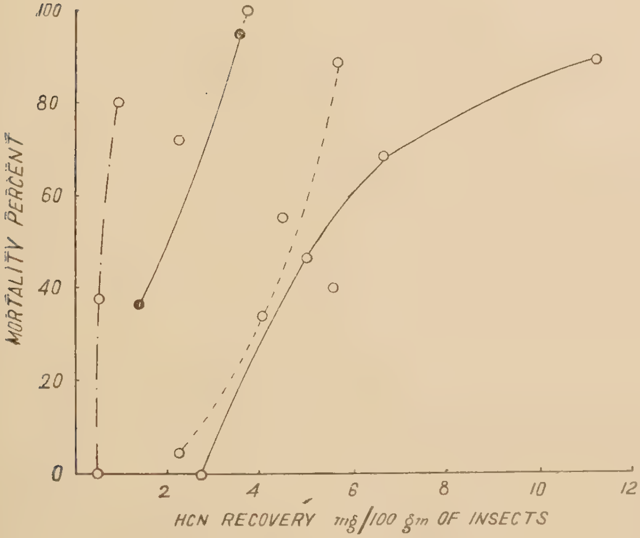

12
10
8
6
4
2
0
RECOVERY OF HCN mg/100 gm OF INSECTS
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
HCN CONCENTRATION mg/LITRE
RECOVERY OF HCN FROM DIFFERENT SPECIES OF INSECTS EXPOSED TO DIFFERENT CONCENTRATIONS OF HCN
[STUDY OF SURFACE RESISTANCE]

Fig. 7.



RECOVERY OF HCN FROM DIFFERENT SPECIES OF INSECTS EXPOSED TO DIFFERENT CONCENTRATIONS OF HCN (HIGHER CONCENTRATIONS) (STUDY OF SURFACE RESISTANCE)

Fig. 8.



RELATIONSHIP BETWEEN MORTALITY AND RECOVERY OF HCN IN DIFFERENT SPECIES OF INSECTS [STUDY OF INTERNAL RESISTANCE]

Fig. 9.

Fig. 6 shows that susceptibility to HCN varies considerably in the different species of test insects. They may be arranged in descending order of their susceptibility as first- and second-instar nymphs of *Drosicha* sp. > third- and fourth-instar nymphs of *Drosicha* sp. > seventh stage caterpillars of *C. cephalonica* > adults of *T. castaneum* > adult females of *Drosicha* sp. The somewhat lower mortality than expected, shown by third- and fourth-instar nymphs of *Drosicha* sp. at 1·03 mg. /litre concentration of HCN, was probably due to variation as there was only one observation at this concentration. Further, fig. 7 will show that the same insects may be arranged in descending order of the quantities of HCN recovered per 100 gm. of body weight thus, *C. cephalonica* > first- and second-instar nymphs of *Drosicha* sp. (at one out of three concentrations, however, the order gets reversed) > adults of *T. castaneum* > third- and fourth-instar nymphs of *Drosicha* sp. > adult females of *Drosicha* sp. The two orders are identical so far as *C. cephalonica*, *T. castaneum* and *Drosicha* adult females are concerned but the nymphs of *Drosicha* sp. do not occupy the same relative positions in the two series.

Recovery of HCN from insects exposed to superlethal concentrations of HCN.

In another series of experiments the insects were fumigated for 30 minutes with much higher concentrations of HCN than was necessary to give 100 per cent. mortality. These are therefore referred to as superlethal concentrations. After fumigation these insects were subjected to exactly the same process of HCN recovery as described earlier. The results are given in fig. 8.

It will be seen from fig. 8 that the order of HCN recovery was much the same as when lower concentrations of HCN were used. The caterpillars of *C. cephalonica* gave the maximum amount of HCN followed in descending order by first- and second-instar nymphs of *Drosicha* sp., third- and fourth-stage nymphs of *Drosicha* sp., *T. castaneum* adults, and lastly females of *Drosicha* sp. Thus here also the order between *C. cephalonica*, *T. castaneum* and *Drosicha* sp. adults is the same as in figs. 6 and 7 but the nymphs of *Drosicha* sp. behaved in a different way.

Recovery of HCN from dead and surviving insects.

For these experiments, two parallel batches of insects were weighed separately and fumigated with a concentration of HCN that was expected to give a mortality in the neighbourhood of 50 per cent. with an exposure of 30 minutes. After the fumigation, the dead from one batch and the living from the other were distilled separately and the amount of HCN recovered determined. The remaining live individuals from the first lot and the dead from the second were weighed immediately so that the difference between the two weights gave the weight of the insects distilled. The results of the experiment are given in Table V.

TABLE V.

HCN recovery separately from dead and surviving insects.

Name and stage of insect	Fumigation at			HCN recovery			
	Temp. °F.	R.H. per cent.	Mean HCN conc. mg. per litre	Dead insects		Surviving insects	
				No.	HCN mg./ 100 gm.	No.	HCN mg./ 100 gm.
T. castaneum adults	92–93	70	1·2	166	8·5	197	2·3
C. cephalonica, 7th stage caterpillars	84–86	—	0·94	25	5·2	27	2·3

In both *T. castaneum* and *C. cephalonica* the surviving insects gave a smaller amount of HCN per 100 gm. body weight than the dead.

Comparison of HCN recovered from and sorbed by different species.

The foregoing experiments, together with observations reported by previous workers, have indicated the possibility of a positive correlation between specific susceptibility and the recovery of HCN per gm. body weight of fumigated insects. This correlation has also been indicated within the same species, as smaller quantities of HCN are recovered from the comparatively less susceptible individuals (surviving insects) than from the more susceptible individuals (dead ones) of the same species. This smaller recovery of HCN from the less susceptible insects (adult females of *Drosicha* sp.) may be due to either (a) less sorption of HCN or (b) conversion of larger amounts of sorbed HCN into non-recoverable substances. In order to throw some light on these two alternatives, an effort was made to estimate the differences between sorption and recovery of HCN. For this purpose the apparatus shown in fig. 3 was found to be suitable.

The sorption chamber (fig. 3) was first partially evacuated to a known pressure and then filled with HCN-air mixture from a reservoir. The total amount of HCN drawn into the sorption chamber was then determined by completely evacuating the chamber by vacuum pump and allowing the HCN air mixture so removed to bubble through one millilitre of sodium carbonate solution diluted to 1·5 ml. in the receiving tube which was kept surrounded by ice. After the first evacuation of the HCN-air mixture from the sorption chamber, HCN-free atmospheric air was let into the sorption chamber to fill it up completely. Again this air which might contain some traces of HCN remaining in the sorption chamber, was allowed to bubble through sodium carbonate in the receiving tube. In this way, the chamber was washed with HCN-free atmospheric air, three times, in order to remove all HCN from the sorption chamber and to get it entrapped in the receiving tube. The HCN thus trapped was estimated by the alkaline picrate method. A number of insects were then placed in the same sorption chamber and the whole of the above process was scrupulously repeated after allowing the insects to remain exposed to HCN in the chamber for 30 minutes. The difference between the first and the second estimations of HCN gave the amount of HCN sorbed by the insects. Further, the insects were removed from the sorption chamber and distilled in the microstill (fig. 2) and the amount of HCN recovered was determined. The results are given in Table VI.

Although it will be observed that there is considerable variation both in the amounts sorbed and the amounts recovered per gram body weight in the three different experiments, it can be seen from Table VI that the weight of HCN sorbed per gram body weight of adult females of *Drosicha* sp. is much less than in the case of *C. cephalonica* caterpillars under similar conditions of fumigation. This would therefore account for the lesser recovery of HCN from the less susceptible adult females of *Drosicha* species unless it were found that a relatively large amount of HCN sorbed by them had been converted into some non-recoverable product. Table VI shows that in each species the amount of HCN recovered is less than the amount sorbed, indicating that part of the HCN is converted into such non-recoverable products ; but this converted amount is less in *Drosicha* adults than in *C. cephalonica* caterpillars. Thus it appears that it is the difference in sorption of HCN and not the difference in the amount of HCN converted, which is responsible for less recovery of HCN from the less susceptible adult females of *Drosicha* sp.

Water content of different species of insects.

The water content of caterpillars of *C. cephalonica*, adult *T. castaneum* and the adult females of *Drosicha* sp. was estimated by first accurately weighing and then dehydrating them by keeping in an oven at 100°–102°C. and afterwards in desiccators

TABLE VI.

Difference between sorption and recovery of HCN.

Name and stage of insect	Number used	Total weight of insects	HCN-conc. in reservoir mg/litre	Vacuum (mm. Hg.) in chamber before taking in HCN-air mixture	HCN sorbed in microgm.	HCN recovered in microgm.	Difference between sorption and recovery in microgm.
C. cephalonica, 7th stage caterpillars :							
I	10	0·158*	4·46	684	78·0 (493·6)	48·0 (303·8)	30·0 (189·8)
II	40	0·98	3·69	510	121·2 (123·6)	49·9 (50·9)	71·3 (72·7)
III	40	1·2093	3·76	510	112·0 (92·6)	40·0 (33·1)	72·0 (59·5)
Drosicha sp., adult females :							
I	16	2·9008*	4·46	684	36·0 (12·41)	33·0 (11·38)	3·0 (1·03)
II	16	2·0812	3·69	510	40·0 (19·2)	19·0 (9·1)	21·0 (10·1)
III	16	1·4008	3·76	510	20·0 (14·3)	14·2 (10·1)	5·8 (4·2)

Note.—The figures in brackets show HCN in micrograms per gram body weight of the insect.

*In this experiment the insects were not actually weighed and these figures indicate the weights calculated from the average weight per insect from the same population. The two last experiments were carried out after a lapse of about 1½ years ; hence the insects used in these experiments were from a different population.

till a constant weight was obtained. The difference between the first weight and the final weight gave the moisture content of the insects. The results are given in

TABLE VII.

Water content of insects.

Insects	Average moisture per cent.
T. castaneum adults	45·39
C. cephalonica 7th stage caterpillars	66·36
Drosicha sp. adult females	42·68

It will be seen that the percentage of water content of the caterpillars of *C. cephalonica* is the highest and that of adult females of *Drosicha* sp. is the lowest. Thus a positive correlation between water content and higher susceptibility to HCN and also greater recovery of HCN per gram body weight is indicated.

Discussion.

Sorption and susceptibility.

Carpenter and Moore (1938) found that " the amount of HCN sorbed varies with the species " and that " those insects which are generally known to be difficult to kill, were found to sorb smaller quantities of HCN than those which are easily killed ". Lindgren and Sinclair (1944) similarly obtained less recovery of HCN from the resistant strain of citrus scale-insects than from the susceptible one. These are the only major works on the sorption of HCN by insects that have been published so far. As will be seen from Table IV and figs. 6, 7 and 8 the experiments reported in this paper corroborate the above findings so far as the caterpillars of *C. cephalonica*, adults of *T. castaneum* and adult females of *Drosicha* sp. are concerned, but the results obtained with nymphs of *Drosicha* sp. do not fit in with the above mentioned generalisation. Hence we are not in a position to make the general statement that insects which are more difficult to kill, sorb smaller amounts of HCN. Probably other factors are also involved. An effort has been made below to analyse this problem.

Analysis of effective resistance.

The present investigation has gone one step further than those of the above-mentioned workers. Carpenter and Moore (1938) and Lindgren and Sinclair (1944) estimated both sorption and recovery of HCN only from insects exposed to superlethal concentrations of HCN, *i.e.*, concentrations which were much higher than those necessary for practically complete mortality. We also did the same but, besides recovering HCN from insects exposed to different superlethal concentrations of HCN, we have also estimated both the recovery of HCN and the mortality percentages from parallel batches of insects exposed together to different concentrations of HCN which gave various mortality percentages ranging from 0 to 100. The data from these parallel experiments have been arranged in the Table IV and plotted in fig. 6 and 7 in the form of concentration-mortality curves on the one hand and concentration-recovery curves on the other for different test insects. The same data have also been rearranged in Table VIII making it possible to correlate directly percentage mortality with HCN recovery, as the estimations of these factors were based on parallel batches of insects exposed together to the same concentration of HCN. These correlations for different test insects are graphed in fig. 9 (p. 409).

It is interesting to compare fig. 6 with fig. 9 which shows quite paradoxical results. Assuming that the amount of HCN recovered from insects immediately after fumigation can be taken as an index of the " internal dose " of HCN, *i.e.*, of the amount

TABLE VIII.

Data from Table IV rearranged to show the relationship between mortality of different species and the amount of HCN recovered.

Insect	Stage	Average mortality per cent.	Average recovery of HCN (mg./100 gm. of insects)
T. castaneum	Adult	5·6	2·2
		33·8	4·0
		55·8	4·4
		89·0	5·6
C. cephalonica	7th stage caterpillars ...	0·0	2·7
		40·0	5·5
		48·0	4·7
		69·0	6·6
		90·0	11·5
Drosicha sp.	1st and 2nd instar nymphs	73·0	2·4
		100·0	3·7
Drosicha sp.	3rd and 4th instar nymphs	37·0	1·4
		96·0	3·5
Drosicha sp.	Adult females	0·0	0·29
		35·0	0·5
		80·0	1·0

sorbed, it can be seen from Table VIII that an amount as small as 1·0 mg. of HCN sorbed by 100 gm. of adult females of *Drosicha* sp. has resulted in 80 per cent. kill, while as large an amount as 6·6 mg. of HCN sorbed by 100 gm. of larvae of *C. cephalonica* has given only 69 per cent. kill ; although the amount of HCN introduced in 100 gm. body weight of *C. cephalonica* was more than six times as much as that introduced in 100 gm. body weight of adult females of *Drosicha* sp., the mortality in *C. cephalonica* was about 11 per cent. less than that in the adult females of *Drosicha* sp. This shows that *C. cephalonica* are far more resistant to sorbed HCN than the adult females of *Drosicha* sp. This conclusion is quite contrary to that arrived at from Table IV and fig. 6, which clearly indicated that the adult females of *Drosicha* sp. were far more resistant to HCN fumigation than *C. cephalonica* larvae. Thus, the order of susceptibilities of these insects to *sorbed* HCN is different from that of their susceptibilities to HCN concentrations in the fumatorium. The descending order of susceptibility of the different test insects to *sorbed* HCN, as shown by fig. 9, is adult females of *Drosicha* sp. > first- and second-instar nymphs of *Drosicha* sp. > third- and fourth-instar nymphs of *Drosicha* sp. > *T. castaneum* adults > *C. cephalonics* larvae. We propose therefore to refer to the amount of HCN recovered as " internal dose " of HCN and to the concentration of HCN in the fumatorium to which the insects are exposed as " external dose " of HCN.

The previous workers, Carpenter and Moore (1938) and Lindgren and Sinclair (1944), did not study the susceptibility of insects to the internal dose of HCN although Carpenter and Moore realized the importance of such a study. They remarked : " Unfortunately the concentration of hydrocyanic acid that will kill 99% of the confused flour beetle (0·5 milligrams per litre of hydrocyanic acid in a 10 minute period) is so low that it is not possible to determine how much hydrocyanic acid is sorbed from this dosage. Consequently we are not able to determine whether or not the internal dose necessary to kill rice weevil is greater than that required to kill confused flour beetles." The observations reported in the present contribution, however, enable us to say for example that adult females of *Drosicha* sp. are most resistant to the external doses of HCN to which they are exposed but that they are most susceptible to the internal dose of HCN, *i.e.*, the amount of HCN actually sorbed. Conversely, the larvae of *C. cephalonica* are most resistant to an internal dose of HCN. This distinct gap between the susceptibilities of insects to *external* and *internal* doses of HCN necessitates the following differentiation. It is suggested that the resistance of an insect shown during practical fumigation, *i.e.*, to the concentration of a fumigant to which it is exposed (external dose) be termed the total " Effective Resistance " while the resistance to the internal dose of HCN, *i.e.*, to the amount of HCN sorbed may be termed the " Internal Resistance ". The adult females of *Drosicha* sp. have shown the highest " Effective Resistance " to HCN fumigation but their " Internal Resistance " has been shown to be the least. Just the opposite is the case with caterpillars of *C. cephalonica*. The difference between total " Effective Resistance " and the " Internal Resistance " can be explained only on the assumption that some other factor comes into play and that that factor is most likely to be the resistance offered by the surface of the insect body to the entry of a fumigant into the body of the insect. Let us call this resistance " Surface Resistance ". It is, at least partially, indicated by the sorption or recovery data. Thus, in the case of female adults of *Drosicha* sp. we can assume that the " Surface Resistance " is so high that it does not allow entry into the body of a sufficient quantity of HCN to cause death, however small that quantity may be. On the other hand in case of *C. cephalonica* the " Surface Resistance " is so low that even at very low concentrations of HCN in the fumatorium a sufficient quantity of HCN is allowed to enter the body to cause death although the " Internal Dose " required to kill this insect is quite high. Thus it appears, in short, that the " Effective Resistance " of an insect to a fumigant is the resultant of its " Surface Resistance " and its " Internal Resistance ".

In the light of the above assumption, the results of the experiments recorded in this paper can be interpreted in the following way. The adult females of *Drosicha* sp., although highly susceptible to internal doses of HCN (*vide* fig. 9), are found to have maximum " Effective Resistance " (see Table IV, fig. 6) ; this is due to their " Surface Resistance " being so high that it completely masks their " Internal Susceptibility ". Conversely, although the " Internal Resistance " of *C. cephalonica* is high, its surface " Resistance " is so low that the resultant is a very low " Effective Resistance ". In the nymphs of *Drosicha* sp., the " Surface Resistance " seems to be higher than in *C. cephalonica* as there is less recovery of HCN from the nymphs (fig. 7), but their " Internal Resistance " to HCN is far lower than *C. cephalonica* (Table VIII & fig. 9). This results in a lower " Effective Resistance " of the nymphs of *Drosicha* sp. than that of *C. cephalonica* (fig. 6). This explains why there was less recovery of HCN from the nymphs of *Drosicha* sp. although they were more susceptible to HCN fumigation than *C. cephalonica*.

The need to analyse the total " Effective Resistance " is not likely to be realised in actual control operations but it is of importance in the search for new insecticides and fumigants on a rational basis. In the past a good deal of work has been carried out to correlate the chemical constitution of insecticides and their toxicity. Some investigators, Haas (1934) and others, have carried out chemical analysis of insects and correlated their chemical content with their susceptibility. In most of this work it is only the total " Effective Resistance " of the test insect to the insecticide that is taken into account and not the " Surface Resistance " and " Internal Resistance " separately. As there are no data to indicate that chemical constitution is likely to be correlated in a similar manner to these two components of the total " Effective Resistance " the undesirability of trying to correlate either the chemical constitution of the insecticide with total " Effective Resistance " of the insect or the chemical content of the insect with total " Effective Toxicity " of the insecticide is evident.

Many workers are concerned in endeavouring to find out out how insecticides act and to correlate toxicity with physiological action. There is indeed implicit in all this such factors as penetration or to use the present phrase " surface resistance " and the complex factors that determine whether an insecticide is to get to the site of action unchanged and how it reacts there, *i.e.*, what we term " internal resistance ". Our main purpose in stressing the points raised is that by re-arrangement of the same set of data in different ways it has been possible, to demonstrate at least partially, these two components of " Effective Resistance".

Water content of insects and sorption of HCN.

Lubatti (1945) observed that " an increase of moisture content of wheat brings about the increase of sorption with all the fumigants studied. Moisture content is a factor as important as time, concentration of vapour or temperature." Bartholomew, Sinclair and Lindgren (1942) working on HCN sorbed by citrus tissues, on the other hand, stated that " there was no significant difference between HCN sorbed by fresh and wilted leaves with a difference in moisture content of 10 to 15% ". During the present investigation, the percentage of water content of *C. cephalonica*, *T. castaneum*, and adult females of *Drosicha* sp. varied in the same descending order as the amount of HCN recovered per 100 gm. body weight. It appears, therefore, that the water content of the insect body may be one of the factors which plays a part in " Surface Resistance " of an insect to a fumigant.

Summary.

The relationship was studied between susceptibility of a number of different species of insects to HCN fumigation and the recovery of HCN from them immediately after fumigation.

The test insects used were *Tribolium castaneum*, seventh stage caterpillars of *Corcyra cephalonica*, first- and second-instar nymphs of *Drosicha* sp., third- and fourth-instar nymphs of *Drosicha* sp. and adult females of *Drosicha* sp.

The apparatus and methods used in the fumigation and in the recovery of HCN from the fumigated insects are fully described.

Preliminary experiments showed that the processes of distillation and redistillation did not affect the recovery of HCN but that the result obtained for recovery from distillation could be affected if some volatile reducing substance were produced and carried over to the distillate. It was found that this did actually take place in the case of one of the test insects—*T. castaneum*—but that redistillation got rid of the impurity.

In the main experiments it was shown that, on the assumption that the concentration of HCN to which insects are exposed is the effective dosage, the susceptibility of the test insects varied in the following descending order : first- and second-stage nymphs of *Drosicha* sp. > third- and fourth-stage nymphs of *Drosicha* sp. > *C. cephalonica* > *T. castaneum* > the adult females of *Drosicha* sp.

When the same insects were arranged in descending order of the quantities of HCN recovered per 100 gm. of body weight, the order was identical except for the nymphs of *Drosicha* sp. which occupied a different relative position. The two categories of nymphs of *Drosicha* sp. were found to occupy a different relative position again with regard to the other three test insects when exposed to a superlethal concentration and assessed for recovery of HCN per 100 gr. body weight.

Parallel batches of *T. castaneum* and *C. cephalonica* were fumigated and the HCN was recovered from the dead and survivors. More HCN was recovered from the dead insects than from those that survived.

Both recovery and sorption of HCN were estimated separately in parallel batches of insects (adult females of *Drosicha* sp. and *C. cephalonica*). Recovery was found to be less than sorption showing that a part of the HCN absorbed is converted into a non-recoverable state. Further, that the weight of HCN sorbed per gram body weight of adult females of *Drosicha* sp. is much less than in the case of *C. cephalonica* under similar conditions of fumigation and that the amount of HCN converted into non-recoverable products is less in *Drosicha* adults than in *C. cephalonica.*

A comparison of the water content of *T. castaneum, C. cephalonica* and *Drosicha* sp. (adults) showed that there was a positive correlation between water content and higher susceptibility to HCN and greater recovery of HCN was also indicated. It is suggested that this may be a factor in the " Surface Resistance " of an insect to a fumigant.

The observations of previous workers that larger amounts are sorbed by or recovered (after fumigation) from more susceptible species than for those less susceptible was corroborated by the results obtained with *C. cephalonica, T. castaneum* and adult females of *Drosicha* sp. but not with those from nymphs of *Drosicha* sp.

When dosage-mortality graphs were prepared by taking the amount of HCN recovered per gram body weight as an index of internal dose, the order of resistance of different test insects based on this new criterion was found to be entirely different from that based on the usual criterion of the concentration of HCN in the fumatorium being the index of effective dosage.

These apparently anomalous observations may be explained by assuming that the resistance shown by an insect in an actual fumigation operation, *i.e.*, to the concentration of HCN to which it is exposed (external dose) is what may be called the total " Effective Resistance " and that this " Effective Resistance " is the resultant of (*a*) " Surface Resistance " to the entry of fumigant and (*b*) " Internal Resistance " to the amount of HCN which actually gains entry into the body in some way or other. Thus the " Effective Resistance " of an insect may be due to a combination either of low " Surface Resistance " and high " Internal Resistance ", giving a very low " Effective Resistance " as in the case of *C. cephalonica*, or *vice versa* giving the maximum " Effective Resistance " as in adult females of *Drosicha* sp. The lower recovery of HCN from the nymphs of *Drosicha* sp., although they were more susceptible to fumigation than *C. cephalonica*, is explained by their higher " Surface Resistance " combined with a very much lower " Internal Resistance ", leading to a lower " Effective Resistance ".

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THE DISCOVERY OF THE EARLY STAGES OF *SIMULIUM NEAVEI* IN PHORETIC ASSOCIATION WITH CRABS AND A DESCRIPTION OF THE PUPA AND THE MALE.

By J. P. McMahon.

The breeding-place of *Simulium neavei* Roub. has remained undisclosed, despite many attempts to discover it, ever since Dr. S. A. Neave first collected the females in 1911.* In May of that year Neave made collections near the Yala River, Kenya, and in November of the same year caught more specimens in Western Ankole, Uganda. Roubaud described and named the species in 1915. Dry in 1921 captured some specimens feeding on man at Ngoina, near Kericho, Kenya, and made the important observation that the local natives connected the fly with the occurrence of a chronic skin disease in this district which has since been identified as onchocerciasis.

Various workers have reported the existence of *S. neavei* in Uganda, Tanganyika and the Belgian Congo, as well as certain parts of Kenya. A so-called new species of Simuliidae was reported from Nyasaland and given the name of *S. nyasalandicum* by De Meillon, but Gibbins (1937) held the opinion that this species was identical with *S. neavei*. Gibbins (1938) reported its existence in Weenan (Natal) and the River Kaomba (Northern Rhodesia).

Efforts to discover the breeding-places have been made by many workers, not merely on the grounds of academic importance but because it is one of the two known vectors of onchocerciasis in the Ethiopian region. The other, *S. damnosum* Theo., occurs in Central and Western Africa but is apparently not concerned with the transmission of this disease in East Africa. All the usual habitats were thoroughly and systematically searched including waterfalls, cascades, stones, sticks and leaves in aerated water, mud, moss, sand, etc., in river beds, river banks of varying texture, trailing grasses and the roots of trees and bushes where they extruded through the wall of the bank. When all the usual habitats had been exhaustively searched, unorthodox places were investigated ; vegetation near rivers and streams was examined for signs of larvae or pupae, and nets were spread over dry and marshy land in order to trap imagines should they emerge. Traps were suspended over streams and rivers for the same purpose but all with negative results. Rivers were dammed and diverted into channels, blasted out of rocky hill-sides, so that cascades and waterfalls could be examined at leisure and in greater detail. Many species of Simuliids were thus collected but *S. neavei* was not found among them.

The successful elimination of *S. neavei* in 1946, from an area in South Kavirondo by Garnham and McMahon (1947), using DDT dripped into rivers, left no doubt that this insect did in fact breed in the fast flowing portions of infested rivers. This knowledge led to renewed efforts to find the early stages and all the probable breeding-places were once again examined but with negative results.

Nets were strung across certain rivers which had been treated with DDT in order to ascertain its effect on fish and other aquatic life. Fish and crabs thus caught were examined for evidence of phoresis but with negative results and it appeared probable that *S. neavei* would be eliminated before its early stages were discovered.

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*It is interesting to note that Capt. C. R. S. Pitman collected crabs (*Potamon niloticus*) in the River Kagera, Ankole, Uganda, in 1928, with larvae and pupae attached to them. Dr. F. W. Edwards, of the British Museum (Natural History), considered them to be a variety of *S. hirsutum* Pom. but one of the original specimens sent to me from the British Museum is undoubtedly *S. neavei*. I am indebted to Mr. P. Freeman of the British Museum who pointed this out to me as I was unaware of this observation at the time of the recent discovery.

First Observations on Phoresis.

During the course of routine observations on fish food at the River Research and Experimental Station, Nyeri, Dr. V. G. L. van Someren noticed an association between mayfly nymphs and Simuliids. At first, in 1948, the association was assumed to be a casual one but later, in 1949, when pupae were also seen in these circumstances, it became apparent that it was of a phoretic nature and the possibility that *S. neavei* might live in a similar state was suggested. This phoretic association was briefly described (1950) and at the same time the discovery of *S. neavei* on crabs was made known. Observations made in Kakamega during 1947 were confined to fish and crabs and it seemed worth while, therefore, to recommence investigations which would include all forms of aquatic life. Accordingly, in February 1950, investigations were begun with this object in view.

Simuliids and Crabs.

Ngoina District, near Kericho, was selected as being most suitable. It is an endemic focus of onchocerciasis and *S. neavei* exists in large numbers. The terrain is typical of such foci ; with small streams dropping precipitously by a series of cascades and waterfalls in heavily wooded country, to the large River Kipsonoi, which at this point flows through deep chasms and has many cascades and waterfalls ; both banks are steep and thickly wooded.

The smaller streams and tributaries were searched in the first instance as *S. neavei* adults seemed to prefer such places. An average of some 2,000 insects were collected and examined daily including Ephemeroptera, Trichoptera and Plecoptera, but no signs of phoresis were observed. Many crabs and a few fish were also collected with similar results. After a period of three weeks spent in this manner, investigations were transferred to the large Kipsonoi. During the second day's search one dead mayfly nymph, *Afronurus peringueyi* (Esb.-Pet.), was collected with a *Simulium* pupa attached to it and, later the same day, three crabs, *Potamon* (*Parathelphusa*) *niloticus* (M.-Edw.), were seen to have live pupae attached to their backs and many others were collected with larvae in a similar position (fig. 1). The imagines proved to be *S. neavei*.

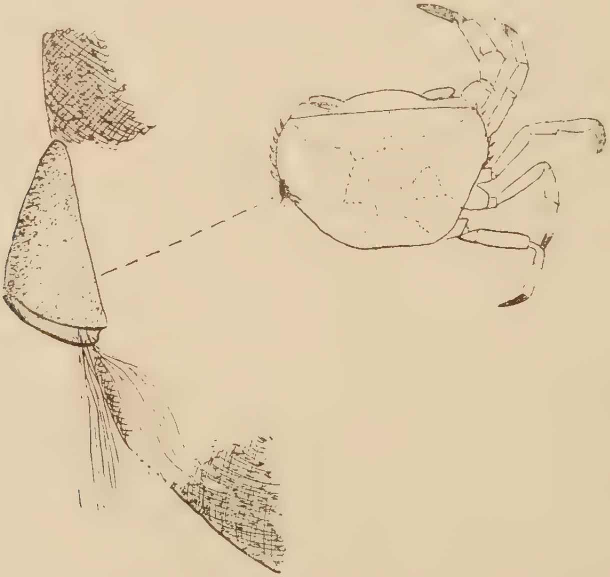

Fig. 1.—Showing pupa of *S. neavei* attached to the carapace of the fresh water crab, *Potamon* (*Parathelphusa*) *niloticus*.

Approximately 50 per cent. of *P. niloticus* were found to have either pupae or larvae attached, usually on the dorsum of the carapace or the basal segments of the legs ; very rarely were they seen elsewhere. In such positions they blend well with the background of reddish-brown and are sometimes difficult to observe ; a casual glance would overlook them. Crabs usually had from one to three larvae on them and the maximum number seen was ten. Pupae usually occurred singly but occasionally two or even three were found on the same crab. Very small larvae were never seen and it is improbable, therefore, that there is direct contact between the female and crab at the time of oviposition.

Technique employed in River Searches.

This survey was instituted mainly with the intention of collecting the aquatic forms of insects and the usual net, constructed of canvas and bolting silk, fixed to a rectangular frame, was employed. It was soon discovered that this type of net was suitable only for rocky river beds, or heavy sand, and that mud quickly clogged the fine meshes, in consequence great difficulty was experienced in collecting insects under these conditions. A box-like trap made of copper wire mosquito gauze built over a stout wire frame, open at one end, was constructed and at the open end a stout pole was fixed so that the trap could be pressed down on to the river bed. Any available stones were then collected and two rough walls constructed, one on either side, so that the current was directed through the trap. Assistants then entered the river at a point 10 or 12 feet upstream and raked the bed with stout poles and with their feet, at the same time walking towards the trap. The force of the current carried most of the insects thus disturbed into the trap and the agitated crabs stalked along with them. The trap was then removed, rinsed thoroughly, and the contents emptied into a large white rectangular enamelled iron tray. Insects were collected with a pipette and the crabs with the aid of forceps. All specimens were examined the same day and those with *Simulium* pupae were isolated in separate vessels for emergence purposes. At first the crabs and nymphs were kept alive, the former in glass jars and the latter in shallow soup plates covered with fine mesh gauze in order to retain any imagines. The number thus obtained was few and it was a slow process limited by the number of vessels available. Most of the pupae were drowned as it was impracticable to aerate water on a large scale under camp conditions. The technique eventually adopted was to kill the crabs and nymphs, remove the pupae and to attach them to strips of damp lint kept in 3 ins. × 1 in. glass tubes with a well-fitting cork. The minimum attention was required and the rate of emergence was increased up to 70 per cent. Water was replenished daily, care being taken not to introduce too much at a time in order to avoid excessive condensation as the insects tend to stick to the side or to drown in even the smallest drop of water in the bottom of the tube.

Habits and Distribution of the Crab.

Three species of crabs were collected but apparently only *Potamon niloticus* is concerned in phoresis. Little is known about the habits and distribution of this crab, which appears to confine itself almost exclusively to rivers and the larger tributaries and is invariably found in cascades and other hard beds, but single specimens are sometimes found in mud. It prefers sunlight and is rarely found in shade ; it is never found in swamps, nor in the smaller streams which tend to dry up during certain periods of the year, although a few specimens have occasionally been found in the last 200 yards of such streams where they have gone presumably in search of food. They are, however, quite often found outside the forest zone in open sunlit streams with but scanty bush. Although little has been done to ascertain its distribution, enough evidence is available to suggest that it will far exceed that of *S. neavei* which is restricted to altitudes between 4,300 and 6,000 feet and cannot exist except under conditions of shade and protection from high winds afforded by forest and wooded rivers.

Other species of crabs appear to restrict their activities to the smaller forest streams where they are found in aerated water but show no particular preference for sun or shade ; they are never found in close association with *P. niloticus*.

Simuliids and Mayfly Nymphs.

Other Simuliids live in a state of phoresis but *S. neavei* is the only one to have been found on crabs. Two species of Simuliids have been bred from pupae attached to mayfly nymphs in Kenya. One of them resembles *S. copleyi* Gibbins and was first observed by van Someren in 1948 at Nyeri. The other is probably *S. lumbwanus* De Meillon as it is indistinguishable from adults trapped by Buckley and described by De Meillon in 1944. Both species were recently collected in the River Kiptiket, a tributary of the Kipsonoi at 6,000 feet, and in the River Sondu at 5,200 feet. At the latter place, *S. neavei* was also found in association with crabs. At Ngoina at 5,200 feet, where the largest number of *S. neavei* was found on crabs, only one damaged pupa was collected attached to a nymph, but this might have been due to temporary conditions and they may be found to occur in abundance during other seasons of the year.

Marlier in the Belgian Congo has isolated two species from pupae attached to mayfly nymphs : these have been described by Grenier (1950). One of them strongly resembles *S. neavei* but certain differences are obvious, namely the claws of the female have a slight tooth and the pupal breathing organ has ten filaments instead of eight. The other is described as a new species and Grenier has named it *S. marlieri* ; it resembles one of the Kenya species from mayfly nymphs (? *S. copleyi*) in the structure of the male genitalia and presence of golden scales on the metanotum.

The available evidence suggests that the *Afronurus*-Simuliid association is more common and has a far wider distribution than that of *S. neavei*-crab.

Eradication of *S. neavei*.

The discovery of the early stages of *S. neavei* will have a profound effect on the work of eradication in the future and the methods employed will have to be modified as a result. Heretofore all rivers and streams were treated with DDT once in every two weeks in chronological order and it will now be necessary to treat only those in which *P. niloticus* is found. Paradoxically enough, disinfestation becomes more complicated in view of the mobility of the crab and it will be necessary to treat all contiguous rivers on the same day, and to include the lower reaches of their tributaries so that dispersing crabs do not escape treatment.

The effect of strong doses of DDT on crabs is not known but the existence of large numbers in previously treated rivers indicates that they are not killed. In the Kodera district, where large doses of DDT, as high as 30 p.p.m., were introduced in 1946, crabs still exist but no larvae or pupae were seen attached to 104 specimens, collected in July, 1950, although other *Simulium* species, including *S. hirsutum* Pom., *S. lepidum* De Meillon, *S. elgonense* Gibbins and *S. dentulosum* Roub. were collected from their usual habitats. It does not appear necessary, therefore, to eradicate the crabs although this may prove to be the easiest course should an effective poison be discovered.

Description of the Male, Larva and Pupa of *S. neavei*.

Male.—Length 3 mm. Wing 2·75 mm.

Head.—Front and clypeus, slate grey, the latter sparsely covered with pale yellow upright hairs. Antenna black and covered with golden pubescence, scape and first flagellar segment brown.

Thorax.—Mesonotum dull black, uniformly and densely covered with golden scales as in female. Scutellum dull black, covered with golden scales and with a fringe of upright golden hairs along the posterior border. Pleura slate grey, lacking patch of hairs on the membrane behind the thoracic spiracle. Halteres yellow with brown stem.

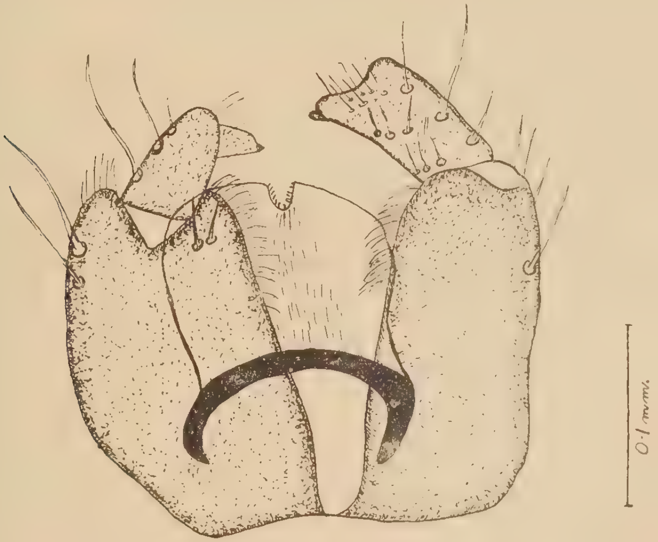

Fig. 2.—Male terminalia of *S. neavei* in ventral aspect. Coxite and claspers are similar to *S. adersi*.

Abdomen.—Dull black; first tergite black with a border of light hairs, some of them very long. Second, fifth and sixth tergites rather densely covered with golden scales and with lateral slate grey patches ; third and fourth sparsely sprinkled with golden scales, the third with lateral tufts of dark hair. Seventh, eighth and ninth tergites dark and devoid of golden scales. *Terminalia* (figs. 2, 3).—Coxites twice

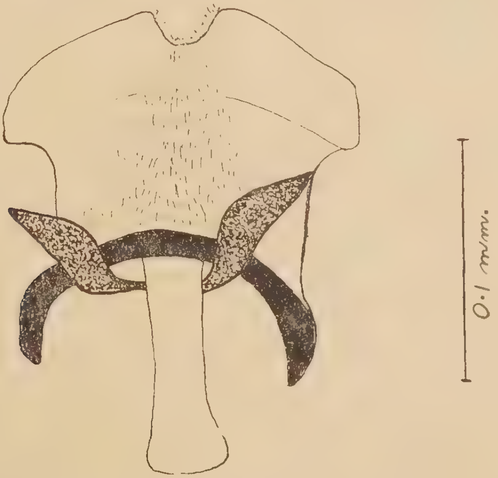

Fig. 3.—Ventral plate of *S. neavei* showing aedeagus extended and the two horn-shaped parameres.

as long as broad, as wide apically as basally and roughly oblong in shape, with long stout setae on anterior distal and ventral surfaces ; clasper about half the length of coxite and as wide apically as basally, with a finger-like process bearing a chitinised tooth. Ventral plate, covered with fine setae, is as broad as long, narrowing medially to give rise to lateral shoulder-like protuberances distally, and with a central semi-circular indentation in the distal edge, basal arms rather long and curved inwardly ; parameres in the form of a pair of strongly chitinised horn-shaped processes. Coxite and claspers similar to those of *S. adersi* Pom. but there are obvious differences in the structure of the ventral plate.

Legs very dark brown and covered with black hairs ; front legs with golden scales on the basal half of the femora and basal two-thirds of the tibia, rather scanty on the former. Middle and hind legs devoid of golden scales except for small patches as the basal tips of the femora. First hind tarsal segment rather swollen ; pedisulcus and calcipala normal. Claws simple.

Wings with basal cell ; about two hairs on the sub-costa and a few hairs on the base of the radius.

Larva.—Length 7 mm. General colour reddish-brown, closely approximating that of the carapace of the crab host.

Pupa.—Length 3·3 mm. Head and thorax smooth and not covered with disc-like tubercles ; trichomes small, slender and simple. Respiratory organ (fig. 4) pale, slender and very long (3·75 mm.), the outer walls are covered with minute pigmented nodules not arranged in rows and the base is covered with disc-like tubercles. The filaments arise from three short main stems dividing into three pairs, two of the filaments again divide at a point less than 0·8 mm. from the base, making eight in all. The respiratory organ is very much like that of *S. hirsutum* but several points of dissimilarity exist. These include disparity in size (*S. neavei* 3·75 mm. ; *S. hirsutum* 2 mm.) ; comparative length of filaments (*S. neavei* five long, three short ; *S. hirsutum* three long, five short, the difference in length being more marked in the latter) and the point at which secondary bifurcation takes place (approximate in *S. neavei* and sub-equal in *S. hirsutum*).

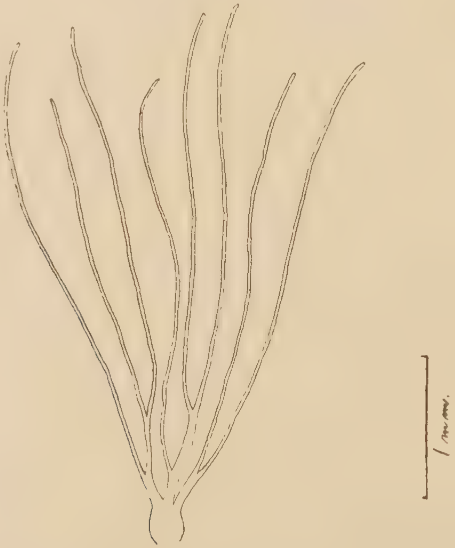

Fig. 4.—Respiratory organ of pupa of *S. neavei*.

The female was first described by Roubaud in 1915 and redescribed by Gibbins in 1937. A brief description is included below in order that, for easy reference, details can be found under a single reference.

FEMALE.—Length 2·5 mm. ; wing 2·5 mm.

Head.—Vertex, front and clypeus slate-grey with pale yellow scales. Antenna black, scape and first flagellar segment brown.

Thorax.—Mesonotum dull black, uniformly and densely covered with golden scales. Scutellum dull black, densely covered with long narrow golden scales and with golden upright hairs along the posterior border. Pleura slate-blue, lacking patch of hairs on the membrane behind the thoracic spiracle. Halteres yellow.

Abdomen dull black, densely and uniformly covered with golden scales on both dorsal and lateral surfaces.

Legs dark brown ; front legs with basal half of femora and outward basal two-thirds of tibiae with yellow scales. Middle legs with basal third of hind femora and basal two-thirds of tibiae with yellow scales ; basal half of first tarsal segment with yellow stripe. Hind legs with basal two-thirds of femora and basal half of tibiae with yellow scales ; basal half of first tarsal segment with posterior yellow stripe. Claws simple.

Wings with basal cell, a few hairs on the sub-costa and hairs on the base of the radius.

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THE ECOLOGY OF EPIDEMIC SLEEPING SICKNESS.
I.—THE SIGNIFICANCE OF LOCATION.

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The survey of an animal or plant population can vary from a simple enquiry into presence or absence to a detailed ecological study, including distribution, frequency, and the geographical features of the areas occupied. But a living population is never static, and the true significance of habitat and geographical range can be brought out only by continued study over a period of years. A short cut may be found to this process if it be possible to trace back the history of a community with sufficient accuracy. The combination of exact geographical and historical information, indeed, is a sound basis for ecology.

An epidemic disease has much in common with an animal or plant population, it is dynamic, exhibiting growth and decay, and consequently it can be profitably studied on similar ecological lines. Complexity is added by the attachment of the causal organism to a definite host, or, in the case of many diseases, to two essential hosts of widely different habits. So the ecology of an epidemic, such as sleeping sickness, is a resultant of the habits of the parasite (the trypanosome) and of its two vectors, the tsetse fly and man. The picture is indeed complex ; yet due consideration shows that in some aspects or at some period one or other of these entities becomes predominant in its influence, giving a degree of simplification which enables us, even with the limited information available, to trace out at least the main influences in the development of an epidemic.

The nature of the parasite in relation to its human host would appear to be the decisive factor in allowing the West African form of trypanosomiasis to attain such huge epidemic proportions, in contrast to the sharp but limited and comparatively brief outbreaks in East Africa. In the *Trypanosoma rhodesiense* infection of East Africa, the patient develops a high temperature and feels very sick as soon as the trypanosome appears in his blood, which is within a week or ten days of the infective bite. He is far too ill to go out into the bush, the habitat of *Glossina morsitans* Westw., the vector ; if untreated he will be dead in a matter of months. Thus the chances of an infected person meeting with and infecting further tsetse are very limited. On the other hand the course of the typical West African form of sleeping sickness, caused by *T. gambiense*, is prolonged and predominantly mild, although fatal. It may be months or years before the patient, or more usually his relatives, realises that he is ill. Untreated cases have been known to survive up to 13 years. With the parasite still active in his blood the infected person will carry on with his normal occupations, such as farming, travelling, hunting and fishing, and this brings him into contact with thousands of tsetse before he is so ill that he will stay at home. Thus the opportunity for each patient to infect the insect vectors, *G. palpalis* (R.-D.) and *G. tachinoides* Westw., is prolonged and may be widespread. Furthermore, the lack of easily recognized symptoms in the early stages of the disease and its imperceptible progress have in the past allowed epidemics to reach considerable proportions before the people became alarmed and tried to escape, in the only way they knew, by abandoning their homes.

In the maintenance of severe local epidemics the habits of the tsetse play an important role. *G. palpalis* and *G. tachinoides* require a habitat with a microclimate of moderate humidities and temperatures. They can neither survive nor reproduce in the dry season climate of the open savanna woodland of West Africa, but are restricted during the six months of adverse dry-season conditions to the favourable habitats afforded by the evergreen fringing forest along the rivers and streams, in other words to the close vicinity of permanent water. But man's requirements, in a country of six months' drought, confine him also to the neighbourhood of permanent water. Here he builds his villages and here he may spend many hours each day. The resulting intimate and regular contact between tsetse and man provides ideal conditions for the transference of infection ; very high rates of sickness and mortality are built up, and this is only checked when the people vacate their villages and move to a new location. The probability is that the new site will be on a river or stream ; there is still contact with tsetse, and, with trypanosomiasis present in the population, a fresh outbreak is inevitable. This can be repeated until the people have been driven right away from the rivers and streams. Such a situation rarely lasts : the dominating need for water is too strong and sooner or later the population moves back to the amenities of the river valley, and the cycle of local epidemics will recommence.

The outstanding feature of the West African epidemics is the extent and severity they have attained. The outbreak in which the present investigations were made covered 60,000 square miles and throughout half of this area infection rates of 5 per cent. to over 15 per cent. prevailed. In the infected parts of Nigeria a population of 1¼ million examined between 1930 and 1935 showed 169,440 cases, or 13·6 per cent. infection. When it comes to a consideration of how these and other epidemics attained such proportions, one is drawn to the conclusion that it is in the habits of the human vector, with his infinitely superior powers of movement, that the answer can be found. The movements of tsetse are extremely limited. Jackson quotes a two-mile movement of *G. swynnertoni* Aust. in three hours as exceptional. *G. morsitans* is reluctant to cross a mile of open clearing. *G. palpalis* is a more active traveller, ranging freely for three to four miles along rivers, but ten miles seems to be the limit of an individual fly's movement. All species can be transported for considerable distances on their hosts or in vehicles, and in Kenya, cattle trypanosomiasis has been spread by means of tsetse carried by trains. But there is no record of the spread of sleeping sickness by artificially transported tsetse, and on theoretical grounds its occurrence would appear unlikely. In contrast with the high infection rates that are found with the trypanosomes of cattle the proportion of flies infected with human trypanosomiasis, even in areas of severe outbreaks, is extremely low, well under 1 per cent. The proportion of a tsetse population that ever gets carried artificially to a distance is negligible. Consequently the chance of an infected fly being transported to a distant locality where conditions are suitable for the establishment of the disease is infinitely small.

In the case of the human vector the exact opposite holds good. It is noticeable that the appearance of some of the most severe and extensive sleeping sickness epidemics followed directly on big increases in movement of native people, for trade, employment or the occupation of new land, which resulted from the pacification and development of Africa by European powers about the beginning of this century. The lack of movement among the more primitive African peoples before this time, owing to the difficulties and dangers of travel, may well have been a major factor in curtailing earlier spread of human trypanosomiasis. It is significant that the first widespread outbreaks known in the Volta River epidemic occurred in the territory of the well governed and stable Mossi Kingdom lying within the bend of the Niger River ; the people themselves being great travellers and having perfect freedom of intercourse and movement within their own country. Further south among the Lobi on both sides of the Black Volta from latitude 9° to 11°, where there was a hotch-potch of small village units constantly at war with one another, outbreaks at the same period remained strictly localised. An old native from this area has told the writer that 50 years ago, before the white man came, if they wished to visit a market four miles away they dared not set forth except with a body of 20 or more well-armed young men. This is a measure of the limitations of travel at that time.

A realisation of the importance of the role played by the human vector in the development of the widespread West African epidemics led to the historical study described in this paper. The conclusions that can be drawn on the ecology of epidemic trypanosomiasis, especially on the type of country favourable for its development and on the main factors operating in its dispersal, have an important bearing on the practical issues of control.

The Location and Spread of an Epidemic.

It seems to have been generally assumed that sleeping sickness originated in and spread from the forest country of the Gulf of Guinea, a country in which four or five species of tsetse are sometimes found together, with *G. palpalis* ubiquitous and common. But an examination of the evidence, both historical and modern, points to this disease as belonging essentially to the dry country of the savanna woodland zone, far to the north of the forest, where *Glossina* is poorly represented and very restricted in range. The earliest record, dating from the 14th century, comes from the upper Niger some 60 miles above Segou where, according to the Arab historian, Ibn Khaldoun, sleeping sickness was very common among the Malli (or Mandingo), attacking especially the higher classes. An unmistakable description of the symptoms is given in connection with the death of Mansa Djata, king of the Malli Empire, in 1374, after two years of illness (Ibn Khaldoun, trans. Baron de Slane 1852–56). Other early references are few and less exact as to locality, but give the same indication. Atkins, in 1734, describes the " sleeping distemper " as common among the negroes of the " coast of Guiney ". It was well known to the slave traders as a fatal complaint among slaves, and the majority of these were, in fact, captured in the interior. Clarke, in 1840, studied cases in Sierra Leone and expressed the view that the " narcotic dropsy " was commoner in the interior than on the coast (Scott, 1939).

The earliest evidence of a precisely determined epidemic known to the present writer comes from Ouagadougou, capital of the Mossi Kingdom, which lies across the headwaters of the three Volta rivers and within the bend of the Niger. It was given by the late Monseigneur Thévernoud, Bishop of the White Fathers' Mission at Ouagadougou, who stated (personal communication) that when he was first there, in 1903, the French doctor, Dr. Allard, recognised a severe outbreak of sleeping sickness and registered numerous deaths at Taessin, eight kilometres north of the capital. The elders of this village declared that an outbreak of the same sickness had devastated the country 35 years previously, that is about 1868. Moreover, the disease was at that time well known to the Mossi people, an intelligent and well organised tribe, under the names " Konoukoungou " or " Ba masgha " (=long sickness). It was said to have been present in their country for a very long time, appearing in a series of short but deadly epidemics which would empty numerous villages. In the intervals between these epidemic waves the disease would die down until, after a number of years, the natives would forget the danger and be drawn back to the homes and farms of their ancestors, which they would rebuild and occupy, whereupon the sickness inevitably re-appeared.

The date of another early outbreak was obtained by the writer from an old native of the village of Tourcoingbam, 150 kilometres north of Ouagadougou and on the headwaters of the White Volta river. A neighbouring village of Punni was almost completely wiped out by sleeping sickness (given under its Mossi name " Ba masgha ") 65 years ago, that is about 1885. He confirmed his date by saying the occurrence was ten years before the arrival of the French in the country, 1895. In two years there were 300 deaths in a population of about 1,000, then the village was vacated. This outbreak presents very interesting epidemiological features which will be referred to later.

About this time the disease was evidently well established on the upper Black Volta river, from latitude 10° 25′ N. to the northern bend, 12° 45′ N. Evidence comes from several sources with dates that correspond closely. Dr. Mackay, reporting on sleeping sickness in the Lawra District in 1924 (Mackay, 1927), quotes local history of an epidemic on the British side of the Volta 40 years previously. This gives a date 1884, but the disease had been known in endemic form on the French side for a longer period. The present writer had similar histories in 1940, from old natives remembering the ravages of the disease (" Kunguon " in Lobi) more than 50 years previously and pointing out ruins along the Volta which had resulted from this outbreak.

Dr. Paul Gouzien made an extensive tour of French Upper Volta and Niger country in 1906–07 and collected data from all sources, from which the following picture emerges. Sleeping sickness was endemic within the quadrilateral Koury-Ouagadougou-Gaoua-Sikasso with a point extending towards Koutiala. The main outbreaks were associated with the Volta rivers, and by 1906 about 120 miles of the Black Volta, from Boromo to Koury, had been almost competely depopulated, the disease having been present here for 20–25 years, that is from about 1880. The epidemic was then (1906) raging along certain tributaries, the most infected villages with populations of 500 to 4,000 showing 80–100 deaths in a year, and the average over a very large area being 5–15 deaths per village annually. Further south, in the Cercle de Gaoua, a more localised but severe epidemic had already caused serious depopulation on the Volta and a tributary, the Bambassou. The death rolls from numerous villages are quoted. An example of the mortality is shown by five small villages with a total population of 1,026, of whom 123 were known to die of sleeping sickness in a year. Dr. Gouzien estimated that 14–15 per cent. of the population of this area were infected annually (Gouzien, unpublished, 1907). A doctor from Gaoua (no name given) in the course of a journey to Ouagadougou in 1905 had remarked on the many villages then devastated by trypanosomiasis, especially along the Black Volta (Father Goarnisson of Ouagadougou Mission, personal communication). Bouffard (1908) mentions three serious centres of endemic sleeping sickness in West Africa :—among the Lobi around Gaoua ; at Koury on the upper bend of the Black Volta ; and in the region Sikasso-Bobodioulasso. Dr. G. Muraz reports that at the time of the arrival of the French at Gaoua, 1899, the disease was prevalent in Volta-side villages, and that the Chiefs and Elders stated that this sickness had been known for a very long time (Muraz, unpublished, 1939). Because of the turbulent nature of the Lobi tribes of this area and the lack of roads there was very little traffic except on the Black Volta, which constituted the main line of communication. The French operated a barge service along the river for revictualling their stations from their headquarters at Koury. This, and the ordinary canoe traffic, aggravated the spread of the disease along the main river and the losses among barge men and canoe men became serious. Koury itself, infected first about 1896, had to be abandoned as a station in 1908 because of the severity of sleeping sickness (records seen at Dedougou, the subsequent headquarters). But the lack of communication among the Lobis confined the epidemics to the close vicinity of the Volta and there was no general spread comparable to that which had occurred among the Mossi, until the activities of the slave raider, Samory, in 1897, started a number of small foci in places where the conditions of human settlements in close contact with tsetse were favourable. But it was not until 1920–25 that severe epidemics began to appear in country away from the Volta, the main lines of extension being up the larger tributaries. From this time on the spread was rapid and the incidence increased alarmingly, more especially in the French Upper Volta Territory to the north and west of the Gold Coast. Colonel Jamot undertook a systematic investigation in this area in May, 1932, and within two years had found 6,905 cases of trypanosomiasis in 111,190 people examined in the Districts of Gaoua and Koudougou alone. This was one of the worst centres of sleeping sickness in West Africa. Out of 347 villages visited by Jamot only 11 were free from the disease. Infection in the villages varied from 2 per cent. to 52 per cent., and the average for the whole area was 6·2 per cent. By the end of 1939 altogether 82,845 cases were known in this area. Wider investigations in 1939 and 1940 by French and British teams showed the huge extent of the epidemic, 30,000 square miles of country, reaching across the Volta rivers to the Niger, with 1,250,000 inhabitants infected at rates varying from 5 per cent. to over 15 per cent. ; lighter infection rates covered an area equally great.

A study of the evidence from the forest and the coast shows that these regions have never experienced anything comparable to the northern epidemics in severity, extent, or duration. In the first place what must be accepted as good negative evidence comes from Bowdich in 1819, who makes no mention of any complaint that could be sleeping sickness although devoting a full chapter in the account of his travels to the sicknesses and native medicines of Ashanti (Bowdich, 1819). Yet in every country where the disease was prevalent a supposed treatment by excising the swollen cervical glands had been developed ; the resulting scars had been noted among slaves from the interior as far back as the end of the eighteenth century. Again, the native attitude towards sleeping sickness where it was known in the forest is very different from that in the infected savanna regions to the north. Kinghorn, working in western Ashanti in 1910, reports that sleeping sickness had been known by the people for many years, if not generations, some very old chiefs having known it since they were children. The testimony was unanimous that it had never been a common disease, never appearing in epidemic form. It was exceptional for more than one or two acute cases to occur together (Kinghorn, 1910). In the same year Horn got closely similar information from the natives of the lower Volta near Anum, that the disease had been known for many years yet it had never produced any epidemic within living memory and formed no part of the natives' traditions (Horn, 1910). A similar story of familiarity with and disregard for this complaint comes from Togoland (Simpson, 1909). This is in marked contrast to the stories gathered from the northern savanna region, where either the natives were fully aware of sleeping sickness as a deadly menace and remembered the devastation it had wrought in the past (*cf.* the Mossi from Ouagadougou, the Grounsi from Koudagou and the Lobi from Lawra and Gaoua) or, as on the Black Volta in the region of Boromo, they feared the vicinity of the river itself and avoided it at all costs, with legends of the water, or the fish, being poisonous (Gouzien, *loc. cit.*). There is even a special provision for sleeping sickness in the divorce laws of one province in Northern Nigeria (Webster, 1909).

When one examines the records from the forest and coast, this indirect evidence is fully borne out. At the time the first observations were made here, 1908–11, sleeping sickness was attracting great attention as an important tropical disease in consequence of the deadly epidemics of Uganda. Moreover, the various observers in the Gold Coast were so struck by the apparent favourableness of the conditions in the forest for the spread of infection and the promotion of epidemics (the universal presence of *G. palpalis* in close contact with villages, the constant traffic along routes crossing numerous fly-infested streams, etc.) that the Government was frequently warned that epidemics were to be expected, additional help was obtained, yet it was rare for more than a few cases to be found after exacting search. The anomaly was so striking that it led to the postulation of theories of acquired immunity (Kinghorn, Horn, and Editor of Sleeping Sickness Bulletin, *loc. cit.*) or that some controlling factor must be at work (Claridge, 1910). It is certain then that any real epidemics could not have been missed. On the coast the disease seems to have been entirely absent. On the lower Volta and in Togoland it was present to a very limited extent, being responsible for at least 112 deaths between 1896 and 1903, with 36 deaths in the latter year, and 63 cases found on inspection of all villages by German doctors in 1908 (Simpson, *loc. cit.*). Yet a six-months' tour of the British lower Volta district in 1909, covering 350 square miles, revealed only two cases in which trypanosomes were found and three suspects (Horn, *loc. cit.*). In the forest region of the Lower Ivory Coast French surveys in 1906 and 1908 showed the absence of any epidemic trypanosomiasis, the few cases found being mainly among soldiers coming from the Soudan (Bouet, 1916). In Ashanti the disease was present at a very low incidence, 16,654 natives of western Ashanti examined in 1911 showing 0·58 per cent. infection (Kinghorn, 1911) and, three years later, 18,768 examinations in north Ashanti revealing only 34 cases, or 0·18 per cent. infected (Ingram, 1914). It was only in the north-west corner that the semblance of an epidemic was found, with a concentration of infection around the three towns Wenchi, Sunyani and Kintampo. In 1910 Kinghorn examined 9,171 natives from this area and found 1 per cent. infected, and Wade, going over the same country a year later, estimated that 1·5 per cent. of the inhabitants were infected but that around Wenchi the rate was 5 per cent. The country around these three north-west Ashanti towns has remained the most heavily infected locality in the forest, although the total number of cases has rarely exceeded 100 in a year. More recently small local foci of infection have appeared in the neighbourhood of Kumasi and along the main roads leading to this centre from the north.

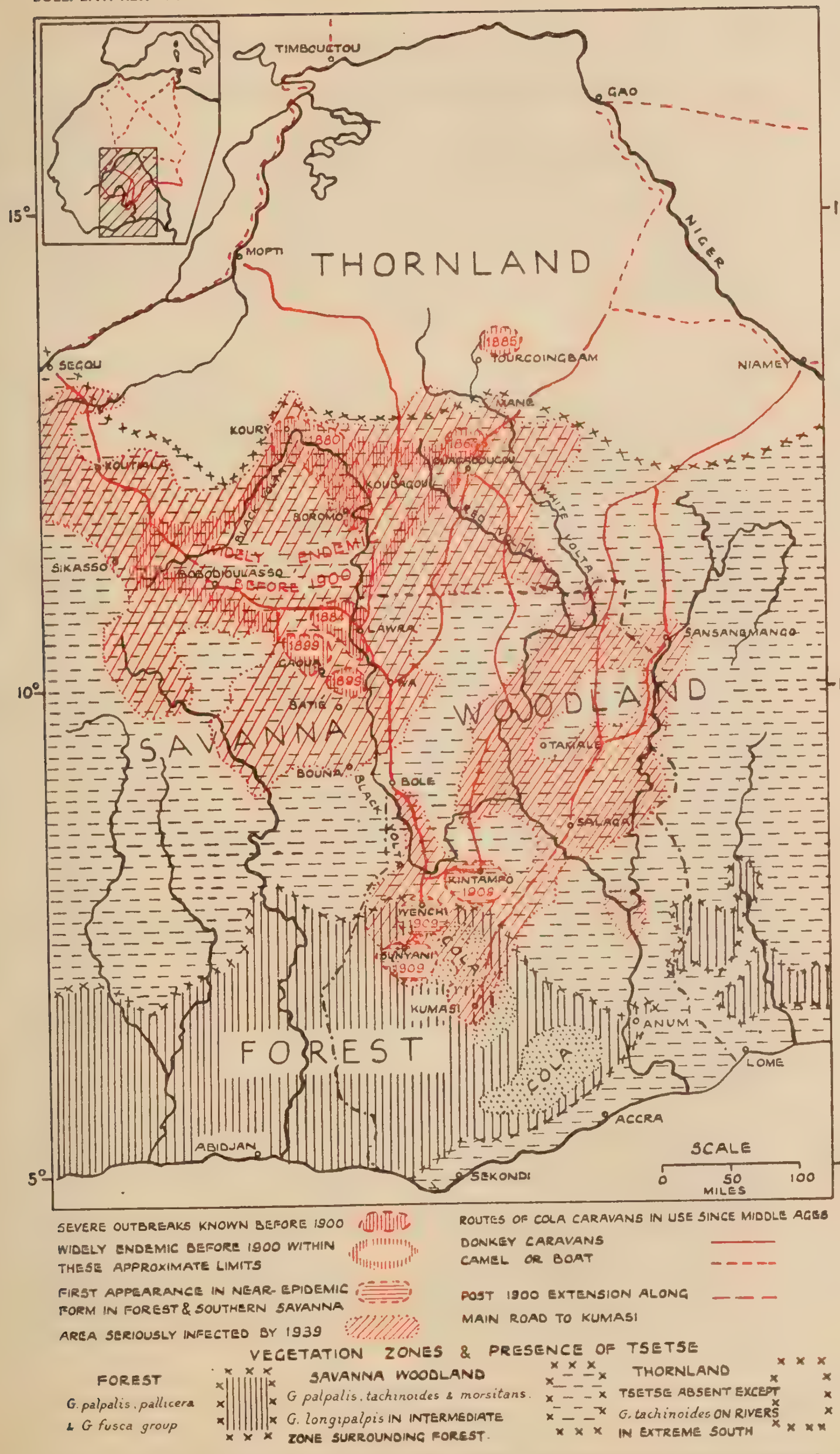
It will clarify the analysis of this mass of data if a brief resume is given of the earlier records of sleeping sickness from Inland Savanna and from Forest and Coast. Map 1, of the Gold Coast and surrounding parts of French West Africa, gives on a reduced scale the information which it has been possible to confirm and locate on larger scale maps. It shows the first definite appearances of outbreaks in northern savanna and in or close to the forest, and the extent reached by the epidemic by 1939, when control measures were started by the French and British simultaneously. The natural features that are included will be discussed in the following section.

Inland Savanna.

1374.	Death of King of Malli from sleeping sickness, which is said to be common on the Niger west of Segou.
1721.	Noticed among slaves from W. Africa, most of whom came from the interior.
1840.	Mentioned from Sierra Leone and considered commonest inland.
1868.	Devastating epidemic at Ouagadougou. Known widely among the Mossi as recurrent epidemic disease with depopulating effects.
1885.	Village near Tourcoingbam on headwaters of White Volta wiped out with 300 deaths.
1884.	Sharp epidemic decimated population of western Lawra District, coming from French side of Black Volta, where known for longer period.
1880–85.	Severe and extensive epidemic on Black Volta north of Boromo ; villages of 800–3,000 population wiped out by 1906.
1896.	Epidemic struck Koury, French Headquarters on northern bend of Volta, abandoned 1908.
1899.	Common in Volta-side villages in the Cercle de Gaoua, known by the people for a long time, but confined to certain localities and only spread after Samory's slave raids, 1897.
1903.	Another epidemic at Ouagadougou with numerous deaths.
1906.	Epidemic raging along Black Volta from Gaoua to Koury. Large villages showing 80–100 deaths a year. Population of Gaoua estimated 15 per cent. infected annually.
1925.	Epidemic spreading to alarming extent and by 1939 3,000 square miles heavily infected country (5 per cent. to over 15 per cent.) and equal area lesser infection extended across upper Volta rivers to Niger, French having 82,845 cases in this area alone.

Forest and Coast.

1850–60.	First appearance of sleeping sickness in Togoland according to German reports, but did not attract attention until 1903, when ascertained at least 112 deaths since 1896, with 36 deaths in 1903.


TIMBOUCTOU
GAO
15°
15°
NIGER
MOPTI
THORNLAND
1885
TOURCOINGBAM
NIAMEY
SEGOU
MANE
KOURY
1880
1863
OUAGADOUGOU
KOUTIALA
KOUDAGOU
BLACK VOLTA
BOROMO
RED VOLTA
WHITE VOLTA
WIDELY ENDEMIC BEFORE 1900
SIKASSO
BOBODIOULASSO
1884
LAWRA
1889
CHOUR
1895
WA
SANSANGMANGO
10°
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WOODLAND
BATIE
SAVANNA
OTAMALE
BOUNA
BLACK VOLTA
BOLE
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KINTAMPO
1909
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SUNYANI
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KUMASI
ANUM
FOREST
COLA
LOME
ACCRA
ABIDJAN
SCALE
0
50
100
MILES
5°
5°
SEKONDI
SEVERE OUTBREAKS KNOWN BEFORE 1900
WIDELY ENDEMIC BEFORE 1900 WITHIN THESE APPROXIMATE LIMITS
FIRST APPEARANCE IN NEAR-EPIDEMIC FORM IN FOREST & SOUTHERN SAVANNA
AREA SERIOUSLY INFECTED BY 1939
ROUTES OF COLA CARAVANS IN USE SINCE MIDDLE AGES
DONKEY CARAVANS
CAMEL OR BOAT
POST 1900 EXTENSION ALONG MAIN ROAD TO KUMASI
VEGETATION ZONES & PRESENCE OF TSETSE
FOREST
G. palpalis. pallicera & G. fusca group
SAVANNA WOODLAND
G. palpalis. tachinoides & morsitans.
G. longipalpis IN INTERMEDIATE ZONE SURROUNDING FOREST.
THORNLAND
TSETSE ABSENT EXCEPT G. tachinoides ON RIVERS IN EXTREME SOUTH

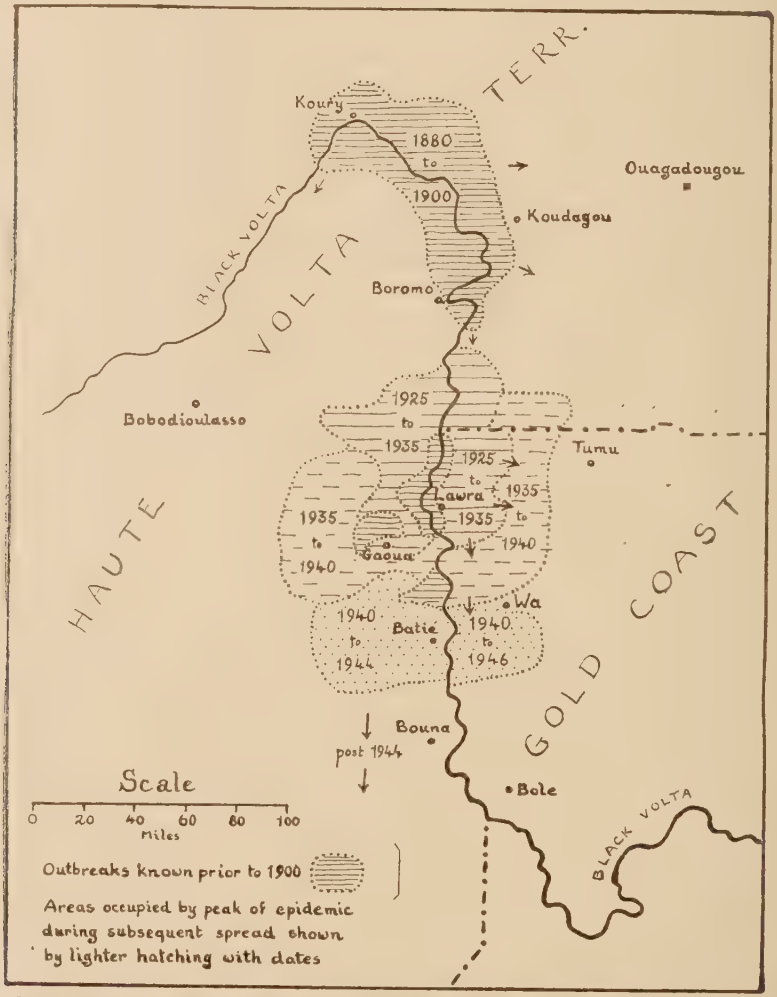
MAP 1. DISTRIBUTION & ROUTES OF SPREAD OF SLEEPING SICKNESS IN THE VOLTA EPIDEMIC.

1901–08. Fifteen cases treated Kumasi hospital, and these came from French territory to the north.
1906–08. Surveys in Lower Ivory Coast showed no endemic trypanosomiasis.
1908. Examination all villages German lower Volta produced 63 cases.
1909. Only two definite cases found British lower Volta.
1910. Ninety-two cases in 9,171 natives examined N.W. Ashanti. Total for Colony and Ashanti was 116 cases by September.
1911. Ninety-seven cases in 16,654 examinations same area, but N.W. quadrant 1·5 per cent. infected, Wenchi 5 per cent.
1914. Thirty-four cases in 18,768 examinations N. Ashanti. Foci of higher infection around Wenchi, Kintampo, Sunyani.

Factors causing the Spread.

Two important features emerge from a study of this information in conjunction with Map 1. The areas of true epidemic sleeping sickness lie in inland savanna country and extend to the northern limit of that zone, with nothing comparable in intensity or duration in the south, although the disease has been present there in a mild form for at least a century. Secondly there is a well-marked relationship between the distribution of infection and the main trade routes which have been in use for centuries, principally by caravans taking the valuable cola nuts from the growing areas in Ashanti to distribution markets in the country of the Niger and the upper Voltas. The three southern towns, Kintampo, Wenchi and Sunyani, where infection first appeared to any extent and which continued to be the most infected localities in or at the edge of the forest, lie at the end of two important cola trade routes coming from the heavily infected areas in the north. A third trade route leads down to Salaga but does not pass any serious northern trypanosomiasis area. It may be significant that no early outbreaks are recorded from this route although it later became an important avenue for the spread of infection. A feature of considerable interest is the extension towards Koutiala of the quadrilateral bounding the original endemic area noted by Gouzien (page 430), and the linking of the Volta epidemic northwestwards with that on the Niger. This distribution corresponds well with the cola route to the big Niger market of Segou ; and that part of the Niger immediately west of this town is the probable location of the earliest known occurrence of sleeping sickness, recorded with the death of the Malli King in 1374 at a place only 60 miles west of Segou.

Now Kintampo, Wenchi and Sunyani are three of the principal markets for the cola trade with the north. They lie in or conveniently near one of the cola growing areas and are the termini for the donkey caravans of the Mossi, Hausa, Grounsi and other traders, who did not penetrate further into the forest because of its narrow and obstructed paths. The importance of this trade is shown by the fact, recorded by Kinghorn in 1911, that the population of Wenchi varied from 3,000 normally, to 15,000–20,000 in the cola season. The trade has probably been in existence since the tenth century ; it was well established when Leo Africanus visited Timbuctoo in 1513. After the cola nuts were bought in the Ashanti markets they were carried by three principal routes, to Kano, to Ouagadougou, or to Segou on the Niger, thence either by boat to Timbuctoo and then on by camel, or direct by camel caravan across the Sahara to the Mediterranean. Here the nuts were worth a dollar each and were sold in exchange for glass beads from Venice or silks from Tripoli. In addition there was a considerable trade in gold, slaves and ivory, linking the upper Niger with the West African forest and hinterland ; and as gold-panning is an old-established activity of the Lobis this may be one of the early links by which infection found its way to the upper Volta from the Malli Empire on the Niger. But by far the most important means of spread would be the cola trade. The traffic is still considerable, but prior to the pacification of the country at the beginning of this century the caravans, for safety and because of the value of their merchandise, numbered from several hundreds to two or three thousand men, complete with advance guards, commissariat, womenfolk and livestock for the journey. The cola is perishable and must be kept moist, which means that the caravans stop regularly at convenient rivers to unpack, sort and wet their loads, and every waterway in the south is tsetse infested. With such large numbers coming from and passing through heavily infected country the continual introduction of infection is inevitable, and ideal conditions are fulfilled for the setting up and maintenance of local foci of sleeping sickness. In this way sleeping sickness could have reached the upper Volta from the Niger, and it would certainly explain the appearance, at the beginning of the present century, of foci of infection in north-west Ashanti from which the disease was gradually disseminated through the forest. Later, the development of mining, cocoa-farming and general trading brought large numbers of labourers into Ashanti from the French Upper


Koury
1880
to
1900
TERR.
Ouagadougou
Koudagou
BLACK VOLTA
VOLTA
Boromo
Bobodioulasso
1925
to
1935
Tumu
1925
to
1935
Lawra
1935
to
1940
1935
to
1940
Gaoua
HAUTE
GOLD COAST
Wa
1940
to
1944
Batie
1940
to
1946
Bouna
post 1944
Bole
BLACK VOLTA
Scale
0 20 40 60 80 100
Miles
Outbreaks known prior to 1900
Areas occupied by peak of epidemic during subsequent spread shown by lighter hatching with dates

Map 2.—The Sleeping Sickness Epidemic on the Black Volta. Showing the location of the limited outbreaks prior to 1900 (only definite records shown), and the subsequent outward spread of infection and southward shift in the main peak of the epidemic. For the sake of clarity the less precisely located dispersal of infection in the area between Ouagadougou and Bobodioulasso is omitted.

Volta and the Northern Territories of the Gold Coast and caused a more general introduction of the disease. The extent to which this took place is shown by the linear distribution of infection along the main motor road (not shown on the map) from Kumasi to Tamale, carrying most of the traffic, which has been noticeable since 1930 (Saunders & Morris, 1932). The southward transference of infection was apparent much earlier, as shown by the treatment at Kumasi between 1901 and 1908 of cases coming from French territory in the north (Simpson, 1909), and Zupitza's observation in 1909 that sleeping sickness was often referred to in Kumasi as "the Prang disease", Prang being a big market for cattle from the upper Volta (Zupitza, 1909). The feature is still in evidence today, that the majority of cases diagnosed in Kumasi and other big Ashanti towns are among labourers coming from the north. Once secondary foci of infection are started up at points along a well-frequented trade route where there is contact with tsetse, the transference of infection, naturally, takes place in both directions. The resulting to and fro movement acts in a most pernicious way, increasing still more the amount of infection in the original endemic areas and building up and maintaining what are really artificially high rates of infection along the trade routes themselves.

Besides this transference of infection, mainly southward from savanna towards the forest, through the medium of travellers, two other main types of spread can be traced (Map 2). In the first place there is an outward dispersal of infection from the original foci which can take place in several ways. The most important of these results from the dynamic nature of an epidemic, which tends to burn itself out in one place, by the removal of the riverside population from contact with the tsetse, but lights up fresh foci in the places to which the people have moved. This form of spread is usually up tributaries and side streams, and leads eventually to concentrations of infection on the smaller streams and headwaters, a typical feature of an advanced epidemic. The movement, however, is slow and limited in extent ; the African farmer is attached to his soil by many ties, real and imaginary, and after a village has undertaken one or two moves, covering perhaps a dozen miles in all, the people always try to return to the location of their original homes. Transport of infection to greater distances can be caused by the emigrant, leaving his village to settle in another part, but movement of this kind is occasional and sporadic, in no way comparable to the steady creep of an epidemic that accompanies depopulation. Another type of local spread results from the incessant movement that is always taking place in an African rural population, to markets, funerals, or just visits to friends, which all helps in the slow dissemination of infection outwards from the focus of heavy epidemics.

The third type of spread, distinct from the two already discussed, is exemplified most clearly in the events of the 1885 outbreak at Tourcoingbam. The devastated village lies on a lake ten miles long, held up by a horseshoe of hills. The country is now bare and open, of a thorn-scrub formation and offering no possible habitat for any species of *Glossina*. But on the lake shore were found one or two examples of the shrub, *Mimosa asperata*, and the tree, *Celtis integrifolia*, both of which, growing in sufficient density, can afford primary habitat for *G. tachinoides*. From the evidence of several natives, this country, at the time of the epidemic, was thickly wooded, it was impossible to follow the lake shores which are now open stretches of reeds, and elephant and buffalo, now found not nearer than 100 miles to the south, were common. It was also impossible to cross the lake on foot because of its depth and width, whereas at present it is regularly crossed in several places. Unquestionably the lake has shrunk and the country around has dried up very much during the 65 years since the epidemic. The present northern limit of tsetse (*G. tachinoides*) is at Mané on the same river 30 miles south of Tourcoingbam, and there was a sharp outbreak of sleeping sickness here in 1945.

This history reveals a very important fact. The agencies which we have so far recognised as responsible for the movements of sleeping sickness have been human. In the Tourcoingbam-Mané events we have a clear example of a southerly movement due to a quite different factor, a change in climate. Now there is ample evidence that a long-term climatic change is in progress, with amelioration of palaearctic climates and, in Africa, increasing desiccation. The result in West Africa is a southward extension of the desert and regression of the forest, and a southward shift in the boundaries of the intermediate vegetation zones, a process which is being amplified by the activities of man (Ahlmann, 1948 ; Bovill, 1933 ; Morris, 1946, pp. 238, 239 ; Wayland, 1940). If the Tourcoingbam-Mané event has any significance, it is to be expected that a similar, slow north-to-south movement would be discernible in the epidemic as a whole. In the area from which we have most complete information, the upper Black Volta, it is quite evident that a gradual southward movement in the location of the maximum amount of infection, the epidemic centre, was in progress up to the time when the application of control measures checked further spread. On the British side the epidemic had passed its peak in Lawra District by 1939, by which time the original locations along the Volta and its bigger tributaries were depopulated and the mass of infection lay along the side streams, especially on their headwaters, indicating the past outward movement of infection away from the main river. The northern half of Wa District (immediately to the south of Lawra) was heavily infected at this time but south of Wa itself the disease was less prevalent. The Lawra and northern Wa outbreaks were brought under control by 1945 but no work could be done in south-west Wa until later. Between 1940 and 1946 an increase in incidence of over 100 per cent. took place in south-west Wa, and villages whose inhabitants had hardly known sleeping sickness ten years before were heavily infected by 1946. At a rough approximation the peak of the epidemic had moved south by 70 miles in 20 years. A similar southward shift of the epidemic can be traced on the French side. The locality of the severe and extensive outbreak of 1880–1900, the bend of the Black Volta, is 100 miles north of Gaoua, which at that time had limited foci only but which, 30 years later, was suffering a widespread and devastating epidemic. Batié, 40 miles south of Gaoua and opposite the heavily infected area south-west of Wa, was showing local increases in infection, despite control measures, up to 1944. Bouna, still further south, was showing a definite increase at this time, and more recent foci of the disease have shown up to the south again of Bouna.

The possibility then must be seriously considered that, besides human agencies, climatic factors have contributed towards a north-to-south movement of epidemic trypanosomiasis. It is not implied that the spread down the Black Volta is entirely due to the effects of climate, the rate of movement is manifestly too rapid. Climate is one component of many factors promoting the spread of infection. But the spread is too directional to have been due to a gradual outward diffusion of infection from original foci, and it is too slow to be accounted for by transference of infection by travellers, a way which would have spread the northern epidemics down the trade routes at a much earlier period had conditions farther south been suitable. This, indeed, is the key to the problem. The environment has been altered in favour of the development of epidemics by the combination of a drying climate and man's activities, the combined effect operating more rapidly than would climate alone.

Before discussing this evidence we must review the possible role of the tsetse in the spread of infection, directly, through its own movements, as distinct from indirectly, through its effect on a population. It is only the individual fly, should it become infected, which is important, and it has already been pointed out that the proportion of tsetse infected with *T. gambiense* is extremely small. The proportion of a tsetse community that moves to any distance beyond a mile or so, either spontaneously or by being carried, is also very small, so the chance of infection being thus spread beyond the existing area of the epidemic is remote. From our studies it does not appear that *T. gambiense* epidemics can be started up at all easily, by the introduction of the odd, isolated infection ; it is the repeated introduction of infections, such as is occasioned by a steady stream of travellers coming from an infected locality, which is effective in spreading the disease. Furthermore it is a fact that epidemic trypanosomiasis arises under conditions where there is the greatest restriction of movement of the tsetse, either intrinsic, as in the case of *G. palpalis* with its marked preference for human food which keeps it in close contact with man, or obligatory, as in the case of *G. tachinoides*, which gives rise to the worst outbreaks in places where its habitat is most limited (Morris, 1949). In other words epidemic areas are the places where the fly is least liable to wander. Logically one must admit that the tsetse, in its local wanderings, could be responsible for a very slow spread of infection, but this amount of movement would be masked by the very much greater and more rapid spread which we have seen can be brought about by man. Compared with the role played by man, the role of the tsetse is not of practical importance in the spread of epidemic sleeping sickness.

Discussion.

The historical and geographical evidence shows that West African sleeping sickness reached the severest epidemic form in the north of the savanna woodland zone and that, despite a continual southward transference of infection by travellers, nothing comparable has developed in the forest. Another interesting feature is the gradual southward shift in the epidemic as a whole, too slow to be due solely to the transport of infection by man, a comparatively rapid affair, but which would appear to be associated with a climatic change towards increasing dryness which is now taking place in Africa. On these two points of great interest further light can be thrown from the evidence of epidemiology.

Sleeping sickness is not a disease that is easily spread, otherwise the whole of tropical Africa, wherever there is contact between man and tsetse, would have been ravaged long ago. The difficulties of spread are mainly intrinsic to the trypanosome ; an infection is not readily taken up by the tsetse, nor is it easily passed on to man by the fly, once infected. In order that the intrinsic difficulties may be overcome sufficiently for an epidemic to develop, a most exacting combination of factors favourable for the exchange of infection is required. These factors are discussed in a previous paper (Morris, 1949) in which it is shown that their combination occurs most fully in the north of the savanna zone and hardly at all in the forest. In the savanna zone the long dry season and xerophytic nature of the dominant vegetation cause a restriction of surface water and of the tsetse's habitat, which usually coincide, and as the local population spend long periods daily at the water-side in the dry season there results a close and continuous contact between tsetse and man. Ideal conditions are thus fulfilled for the transference of the trypanosome and the building up of high infection rates. Restriction of movement of a tsetse is a vitally important factor in making a fly dangerous as a vector. The fly that is free to wander, as in the apparently extensive habitat of the forest, may never bite another person even if it does pick up an infection. The fly that is confined to the narrow habitat fringing a northern water-hole cannot avoid repeated contact with man, which gives it the maximum chance of becoming infected in the first place and ensures that it will then be a menace to all who are visiting this particular spot. Restriction of movement, with the resultant increase in danger, is amplified the further north one goes until the limit of distribution of tsetse is reached. In other words, conditions that are adverse to the tsetse are favourable for the development of epidemic trypanosomiasis. Hence it is found that high rates of infection occur right up to the northern limit of fly. Now if a gradually changing climate is causing a southerly withdrawal of the tsetse, the peak of the epidemic would be expected to show a southward movement also, a shift that is, in fact, found to be taking place. But climate is not working alone, its effects on vegetation can be greatly amplified by the activities of man and his stock wherever there is a heavy population. So, in the well populated parts of northern savanna a restriction of the tsetse's habitat has taken place in a zone of considerable depth, which has resulted in a comparatively rapid southward extension of the zone favourable for the development of epidemic trypanosomiasis. It must also be borne in mind that changes in the homogeneity and climate of the forest resultant on both this major climatic change and man's activities, will work in the direction of a restriction in the habitat of forest tsetse, a tendency that will promote rather than hinder the development of epidemic sleeping sickness there. It is noticeable that the highest infections within forest country occur either along the northern edge, most exposed to the savanna climate, and especially to the dry harmattan wind, or around the centres of man's greatest activity, such as Kumasi.

It is now necessary to examine the practical bearings of ecology on control. The earliest undertakings for the control of sleeping sickness, the evacuation of populations from large areas around Lake Victoria and in the Western Rift, resulted from a focus of attention on the importance of the human vector. The dangers of applying such first-aid measures, without regard to the ecological aspects of the problem as a whole, have been pointed out by Ford in tracing the serious repercussions on the agricultural economy of the country which have resulted from these evacuations (Ford, 1950). A wider approach to the problem and a greater knowledge of the biology of tsetse, especially concerning the control of *G. palpalis* and *G. tachinoides*, now make it possible to evacuate the tsetse instead of man, who is left, untrammelled by the dangers of trypanosomiasis, to consolidate the gains of the entomologist by the full development of the formerly fly-infested country. But there are limits, both economic and biological, to what can, or should, be done in the way of tsetse eradication. In much of West Africa 80 per cent. to 90 per cent. of the country is infested with tsetse. Profound thought, and the consideration of every possible aspect and repercussion of one's activities, must be given before putting forward plans for tsetse control under such circumstances. It is only as a vector of pathogenic trypanosomiasis that the tsetse is a danger. A vital question is the matter of deciding *when* and *where* it is necessary to eradicate the tsetse, and the lengths and expenses to which one is justified in going in order to attain this end. These decisions can be made only on the sound outlook of ecology.

A campaign for the control of West African sleeping sickness should concentrate, first and foremost, on the complete elimination of the disease in the true epidemic centres in savanna woodland. Tsetse eradication offers the most effective and certainly the most stable means of attaining this end, provided it is planned not only on the distribution of high infection rates but on geographical and biological considerations also. Operations can thus cover natural ecological units which are, typically, the river systems. The choice of natural working units is fully justified when consideration is given, as it must be in the properly planned campaign, to the wider benefits to an agricultural population deriving from their being in possession of ample well-watered land free from tsetse. There are many aspects to this side of the problem, but only two will be mentioned here. In the first place the occupation of the river valleys by a farming community engaged in a stable form of agriculture, which must be based on mixed farming and the possession of good livestock, is the best means of ensuring permanence in the reclamation. Secondly, the possibilities of a more active agricultural development, with the raised standard of living that this implies, is itself a justification for the full-scale eradication of the tsetse.

Peripheral to the true epidemic areas, or radiating out from them along trade routes, are secondary areas of much lighter infection. On epidemiological grounds it appears that the secondary areas are offshoots of the epidemic centres and are dependent on them for the continued maintenance of infection. Clearing up the main epidemics should, then, result in a fall in incidence in the secondary areas, where control might then be established by quite local measures for protection from the fly or treatment of cases. There is the possibility that the disease in these peripheral areas might disappear spontaneously with the general lowering of the amount of trypanosomiasis in the country. On this question it is impossible to generalise, so much depends on local conditions.

There remains the big question of the necessity for tsetse control in the forest. There has been ample opportunity, to our certain knowledge since the middle of last century and in all probability much longer, for severe epidemics to have been started up in the forest through the agency of the northern cola caravans, which must have been continually bringing infected people into contact with tsetse-ridden communities in the south, where conditions *would appear* ideal for the promotion and spread of infection (*cf.* Kinghorn, Horn, Claridge, etc.). And infection has been there, but always in a mild degree, and it has never given rise to epidemics in any way comparable to those which developed in northern savanna. The conclusion must be that the forest is, in fact, inhospitable for the continued development of trypanosomiasis and that the low state of endemicity occurring there, and conspicuously centering on some market or trade route connected with the north, is maintained by the constant renewal of infection by introductions from the true epidemic centres in savanna. This is a practical point of the highest importance. Tsetse control in the forest will, admittedly, be difficult and very expensive. Nor can we, with our present knowledge, say that it would be justifiable. So many other factors of possibly greater importance than trypanosomiasis are involved. Besides its intrinsic value for timber and those crops that grow only in shade, the forest has a greater and imponderable value to the country for its influence on soil, water and also climate. But the forest itself constitutes the habitat of the tsetse that live there, and control by clearing would mean literally the removal of the forest. Even control by insecticides, should a practicable application be found, would involve the danger of a vast disturbance to the ecological balance of the fauna and flora, since it cannot be expected that tsetse alone would be killed. But if our arguments are valid we do not need to undertake this task. It should be sufficient to cut off the introduction of infections into the forest, by complete control of the disease in the true epidemic areas, and the disease would eventually die out. But it must not be lost sight of that developments, increases in population, in cultivation and in traffic, will work in favour of the local transmission of infection by causing a restriction of the tsetse's habitat and bringing it into closer and more frequent contact with man. Even if complete control of the tsetse is unnecessary, protective and curative measures have an important role to play in those places where a real danger might arise.

It might be thought that the relationship between sleeping sickness and forest just described for West Africa does not hold for the conditions of equatorial forest, in view of the epidemics that swept the Cameroons and Congo between 1920 and 1940. In fact there is a great deal of consistency in the course of events in these two very different regions. The earliest appearance of the disease in the Congo basin comes from the savanna country of the middle Ubangui River, in the very locality which is the terminus of the caravan routes linking West Africa with the Congo by way of Chad and Archimbault. In 1905 the sickness was only lightly endemic and did not extend further than Mokanga, which is well to the north of the forest (Rodhain, 1907). The natives said that this sickness had been there before 1886 and had spread down from the north. Observations in 1909 made particular reference to the spread of infection along caravan routes coming down to Madingou on the Ubangui, infections being rare just off a route while they were common on it (Martin, Leboeuf & Roubaud, 1909). When the full epidemic had developed, about 1930, it did indeed spread into the zone of tropical forest, where extensive areas of high infection were recorded. But a close examination of the data and maps reveals two important details. In the first place the heaviest outbreaks (the largest numbers of cases among big populations) did indeed occur in savanna or at the edge of the forest. Secondly, in the true forest the population is very sparse, with a density usually below 5 per square mile, and is concentrated around a few centres or along the communication routes, which are very frequently the waterways. It is these particular places which are highly infected (giving the whole region a high infection rate on paper), a circumstance which would be expected for the reasons already brought forward. Finally, it is noticeable that in the more extensively forested area of the Belgian Congo the disease invariably showed much lighter infection rates than those found on the French side north of the river.

Lest it be thought that this approach to trypanosomiasis is too theoretical, it is appropriate to refer briefly to the practical results now visible in the Gold Coast where the plan of control just outlined is already in application. The most serious of the northern epidemics, along the upper Black Volta has been brought under a high degree of control by a combination of mass treatment of cases and eradication of tsetse by selective clearing. There has been a close co-operation between the French and British organisations throughout this work. Recent data on the British side show a 99 per cent. reduction of sleeping sickness in the first 1,100 square miles of country dealt with, Lawra district, where tsetse were eradicated by 1945, and an 85 per cent. reduction in 1,300 square miles of Wa district, where a rising epidemic was still active in 1947 and the work of tsetse control is not yet quite complete. The virtual elimination of sleeping sickness from Lawra district was already in 1949 having a pronounced effect in lowering the incidence of the disease in the adjacent territory where tsetse were still uncontrolled ; a reduction of 98 per cent. occurred between 1939 and 1949 in a strip of country with a population of 7,000 along the eastern boundary, and a reduction of 89 per cent. took place in the same period in villages totalling a population of 7,250 along the southern boundary. Equally valuable is the evidence coming from the trade route passing southwards through Wa and Bole to Wenchi. Regular examination for trypanosomiasis at the French frontier posts has shown a big reduction in the infection rates of travellers returning from the Gold Coast during the past four years. In 750 square miles of country along this trade route in the Bole area, well to the south of our area of control, the examination of 5,000 people in 1950 showed only two infections with trypanosomiasis. And round Wenchi, where the trade route enters the forest, a big decline in the infection rate has taken place recently, from 2 per cent. in 1944 to 0·1 per cent. in 1950, a decline which can be accounted for only by the high rate of control of the disease in Lawra and Wa districts through which pass practically all travellers entering Wenchi. No comparable decline has occurred on the eastern trade route which passes through the still incompletely controlled north-east epidemic area (Saunders, unpublished, 1950). In neither Bole nor Wenchi districts have any measures been taken against the tsetse ; reliance has been placed entirely on the principle of sterilising the main sources of infection in the true epidemic centres to the north.

The collection and interpretation of data on a sufficiently broad basis has made it possible to plan the eradication of sleeping sickness from a country as a practicable and economic proposition. The undertaking also represents a large scale experiment designed on the arguments of ecology. The results that are accruing indicate that the arguments are sound.

Summary.

A combination of historical, geographical, and epidemiological studies has given sufficient insight into the ecology of sleeping sickness to enable the main factors influencing the development and spread of an epidemic to be traced.

The evidence shows that in West Africa sleeping sickness is not primarily a disease of the forest, where tsetse flies are most abundant, but belongs essentially to the dry country in the north of the savanna woodland zone, where the earliest occurrences and severest outbreaks have been located.

The first mention of sleeping sickness comes from the upper Niger and dates back to the 14th century. By the beginning of the present century intense though localised epidemics were devastating parts of the Mossi, Grounsi and Lobi country of the upper Volta rivers. At this time the disease was unknown on the coast and of sporadic occurrence only in the forest. A severe trans-Volta epidemic covering 60,000 square miles, developed between 1924 and 1940, but was confined to the north of the inland savanna zone with nothing comparable in the forest.

The epidemic spread in three principal ways : (1) Outwards from original foci of infection because of the dynamic nature of the disease. This produced a concentration of infection around headwaters, a feature characteristic of advanced epidemics. (2) Through the agency of travellers, originally from north to south but subsequently in both directions : a rapid method of spread producing linear distribution of infection along trade routes. The tempo was greatly increased on the pacification and development of West Africa after 1900. (3) A gradual southward shift in the main epidemic zone appears to be resulting from a long-term change in the African climate which is combining with man's activities to produce a southerly extension of xerophytic vegetation types and a regression of forest.

The most important spread was that caused by the trading caravans, more especially the cola traders, who have been coming down to the cola-nut areas in the Ashanti forest from the big markets on the Niger and Upper Volta since the 11th century. The caravans were formerly very large, up to one or two thousand strong, and were frequently made up of Mossi and Grounsi from the territory that was so heavily infected by the beginning of the present century. It is certain that a continuous introduction of infection would have been taking place into the forest ever since trypanosomiasis was prevalent in the north, that is for 100 years at least. And infection has been known in the forest for about that period, yet always to a mild degree, never reaching epidemic form. It has been sought for, because conditions in the forest, with the vector *Glossina palpalis* in contact with every village and path, appeared to be ideal for the transmission of infection and this drew the particular attention of the early workers from 1908 onwards. But the most that could be found was a threatened epidemic in north-west Ashanti, very significantly centering on the big cola markets which formed the termini for the northern traders.

This historical evidence and the reasoning from epidemiology lead to the conclusion that conditions in the forest are not conducive to the development of epidemic sleeping sickness and that the low state of endemicity found there is maintained by the constant introduction of infection from the true epidemic areas in northern savanna.

From this conclusion arises a practical point of the greatest importance. If the sources from which infection is introduced into the forest could be eliminated the disease there should eventually die out and the tsetse, from the human point of view, would be harmless. Tsetse control in the forest may prove difficult and expensive, and if it is attempted by clearing this might end in the literal destruction of the forest. Such measures would be hard to justify, so many other factors of possibly greater importance than trypanosomiasis are involved, both the intrinsic value of a forest for its products and the wider value through its influence on climate, soil and water.

In formulating a plan for the control of sleeping sickness, the habits of both vectors, human as well as insect, should be considered. The tsetse plays a major role in the development of the high infection rates characterising the epidemic outbreaks in northern savanna ; the human vector distributes infection from these sources along trade routes and into the forest. The elimination of the disease at its source, in true epidemic centres, which can be most effectively accomplished by eradication of the tsetse, will check the distribution of infections to the secondary areas of lighter infection which could then be cleared up by quite minor control measures or might even disappear spontaneously.

This plan is now in operation in the Gold Coast. The validity of the arguments on which it was based is being shown by the results that are already apparent : the high rates of reduction in the epidemic areas and the pronounced lowering of infection in neighbouring, uncontrolled areas, more particularly in the forest region of north-west Ashanti where it is entered by a trade route coming from the previously heavily infected country.

Acknowledgements.

It is a pleasure to acknowledge my indebtedness to Médecin Général Le Rouzic and his successor, Médecin Colonel Jouchère, and to their staff in the Service Général d'Hygiène Mobile et de Prophylaxie of French West Africa. The generous way in which they have given access to information and their ready hospitality, with its accompaniment of lively discussion, have contributed greatly to the study described. I am also grateful to the Medical Department of the Gold Coast for their interest and help in the loan of literature from the Research Laboratory at Korle Bu.

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STUDIES ON BEETLES OF THE FAMILY PTINIDAE.*

V.—The Oviposition Rate of *Ptinus tectus* Boield. under Natural Conditions.

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During the course of an investigation of the ecology of *Ptinus tectus* Boield. in a warehouse (Howe, 1950), an attempt was made to measure the changes in the rate at which eggs were laid in the warehouse over a period of two years. This work was continued at the laboratory in an unheated building when it could no longer be carried on in the warehouse.

Methods.

An estimate of oviposition rates was obtained by placing food "traps" at various places on the floor of the warehouse. About 40 cc. of food was put on a piece of newspaper 31 × 23 cm., left in the warehouse for a week or a fortnight and then replaced by a new sample. After exposure in the warehouse, the samples were collected in small jars and kept at the laboratory for two to four weeks at 25°C. and 70 per cent. R.H. Occasionally an adult beetle was seen on the newspaper. All trap-foods were examined in the warehouse to ensure that no adults were brought to the laboratory in the food sample.

Very few large larvae were found in the food samples after incubation, indicating that little, if any, larval migration into the food had occurred. The number of larvae bred from a food was, therefore, accepted as the effective oviposition rate at that site. The percentage hatch is assumed to remain constant through the whole period.

Warehouse Results.

When the work started in 1945, Canadian vitaminised flour was stored in sacks in the warehouse. In one room, the traps were all baited with the same flour and were placed singly in scattered positions. Few eggs were laid in these traps. Those placed at the edge of the flour stack, where adult *Ptinus* were most abundant, received most eggs ; many of those put between sacks nearer the centre of the stack were not laid in at all.

These traps, being baited with the food already in store, obviously did not attract ovipositing females.

May to September, 1945.

In another room, the traps were placed on the floor in two positions just outside the stack, one very close to a wall (outer site) and the other nearer the middle of the room (inner site). At each site four traps were placed baited with Canadian vitaminised white flour, wholewheat flour (=ground grain), yeast and fishmeal. The four foods were interchanged in position, period by period.

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TABLE I.

No. of larvae bred from oviposition trap foods.

A

May to September, 1945

Trap food	From To	15.v 22.v	22.v 29.v	29.v 5.vi	5.vi 19.vi	19.vi 3.vii	3.vii 17.vii	17.vii 31.viii	31.vii 23.viii	23.viii 4.ix	4.ix 18.ix	18.ix 1.x	Totals 20 weeks
Outer site :													
Fishmeal	...	13	8	2	7	6	41	0	3	12	57	95	244
Yeast	...	23	11	23	2	9	29	0	2	0	1	3	103
Wholemeal flour ...	...	1	10	2	14	5	32	0	24	1	17	47	153
Canadian flour ...	...	1	7	1	5	1	3	0	3	15	6	7	49
TOTAL ...	...	38	36	28	28	21	105	0	32	28	81	152	549
Inner site :													
Fishmeal	...	3	3	8	4	0	5	3	4	88	3	17	138
Yeast	...	7	14	6	27	23	8	1	35	0	2	6	129
Wholemeal flour ...	...	3	24	0	6	4	15	2	6	0	1	5	66
Canadian flour ...	...	0	2	3	1	0	2	0	2	2	1	7	20
TOTAL ...	...	13	43	17	38	27	30	6	47	90	7	35	353

B

September, 1945 to February, 1946

Trap food	From To	1.x 15.x	15.x 29.x	29.x 12.xi	12.xi 26.xi	26.xi 12.xii	12.xii 2.i	2.i 23.i	23.i 6.ii				Totals $18\frac{1}{2}$ weeks
Both sites :													
Fishmeal	...	60	25	74	2	0	0	32	0				193
Other foods ...	...	28	37	54	50	0	20	0	2				191
TOTAL ...	...	88	62	128	52	0	20	32	2				384

C — February to June, 1946

Trap food {From / To	6.ii 18.ii	18.ii 5.iii	5.iii 19.iii	19.iii 2.iv	2.iv 16.iv	16.iv 30.iv	30.iv 21.vi	21.v 3.vi	3.vi 20.vi			Totals 19 weeks
Outer site :												
Fishmeal	0	8	0	59	66	69	131	104	56			493
Wheatfeed	0	0	0	16	14	30	32	59	100			251
Wholemeal flour	1	0	0	17	19	53	7	5	10			112
Canadian flour	13	20	0	14	42	16	3	32	14			154
TOTAL	14	28	0	106	141	168	173	200	180			1,010
Inner site :												
Fishmeal	0	0	0	40	29	51	50	93	82			345
Wheatfeed	0	0	0	1	1	13	36	83	44			178
Wholemeal flour	0	0	0	8	8	15	13	8	34			96
Canadian flour	0	5	0	11	15	15	8	29	10			93
TOTAL	0	5	0	60	63	94	107	213	170			712

D — June to October, 1946

Trap food {From / To	20.vi 2.vii	2.vii 17.vii	17.vii 31.vii	31.vii 14.viii	14.viii 28.viii	28.viii 12.ix	12.ix 25.ix	25.ix 8.x	8.x 10.x			Totals 16 weeks
Outer site :												
Fishmeal	65	78	111	298	227	177	136	94	3			1,189
Wheatfeed	82	135	88	34	183	153	78	132	46			911
Wholemeal flour	20	9	41	29	44	55	25	13	6			242
Cotton seed meal ...	77	150	88	227	348	279	274	504	207			2,154
New copra meal	—	—	—	68	174	95	76	91	34			538
Conditioned copra meal	31	32	28	79	51	180	83	63	19			566
Canadian flour	26	7	—	—	—	—	—	—	—			33
TOTAL	301	411	356	735	1,027	919	672	897	315			5,633
Inner site :												
Fishmeal	33	67	88	204	176	93	79	85	41			866
Wheatfeed	123	23	57	71	168	124	166	146	50			928
Wholemeal flour	25	30	26	16	43	11	100	18	6			275
Cotton seed meal ...	51	118	40	233	324	569	372	301	85			2,093
New copra meal	—	—	—	92	201	111	74	87	12			577
Conditioned copra meal	16	14	13	71	285	249	89	112	31			880
Canadian flour	27	7	—	—	—	—	—	—	—			34
TOTAL	275	259	224	687	1,197	1,157	880	749	225			5,653

These four foods were used for 11 consecutive periods covering a period of 20 weeks from May to September, 1945. Fishmeal was the most attractive food receiving 42 per cent. of the eggs laid in the traps ; white flour received only 8 per cent. The site near the wall received 55 per cent. more eggs than the inner site (Table I).

The results from period to period were very irregular. The very small figure in late July corresponds with a falling off of the apparent numbers of adults and with a period of very low humidity. Otherwise, the results follow the general trend of temperature.

September, 1945 to February, 1946.

After September various other foodstuffs such as dried egg, dried milk, dehydrated potato and soya meal were tried in traps until early February. Not one of these was as attractive as fishmeal which was used throughout this period. Some egg laying occurred throughout the winter, except during a cold spell just before Christmas. The stored flour was finally removed on 4th December and the room was thoroughly swept. The only food available for eating and oviposition after that date was that in the traps and the wastage collected in the cracks in the floor. Consequently the 54 eggs laid in the traps in December and January may represent a large proportion of the total eggs laid in the room over this period.

February to June, 1946.

The room was not used again for commercial storage, so in January, 1946, 50 small experimental bags containing a total of 75 kg. of wheatfeed were placed in the room. The inner trap site was moved to the centre of the room some four feet from the experimental bags. The four foods used in the traps over the 19 weeks to mid-June were white flour, wholewheat flour, fishmeal and wheatfeed. Again the rate of egg laying was low during cool weather, stopped altogether during a cold fortnight in March, and then increased rapidly when the temperature rose. Once again, fishmeal was the most attractive food with 49 per cent. of the total eggs laid in traps ; white flour received 14 per cent. Wheatfeed, the food available in the experimental bags, received 25 per cent. of the eggs laid (Table I). On this occasion the outer site received 42 per cent. more eggs than the inner site.

Very many more eggs were laid in traps in 1946 than in 1945 because the only alternative oviposition site was 75 kg. of wheatfeed in one small floor area as against many tons of white flour covering the entire floor in 1945.

June to October, 1946.

In June, ground cotton seed cake and ground copra cake were added making six traps at each site. When supplies of it finished in July, white flour was replaced by a second sample of ground copra cake. The first sample of copra meal had been found heavily attacked by *Ptinus* and, perhaps for that reason laboratory tests showed it inferior to the second sample as judged by the speed of larval development. These foods were used until the warehouse was vacated in mid-October.

The rate of egg laying in traps over these 16 weeks was six times as great as the previous comparable period, and reached a maximum over the month between mid-August and mid-September. The final samples were in the warehouse for only two days in October but 540 eggs were laid in them. The mean of 270 eggs per day is much above the mean of 160 per day for the fortnight peak period in late August. It seems likely that this difference is due to some attraction of clean food as an oviposition site because, in the laboratory, provision of fresh food generally accelerates egg laying. On the other hand, there was no appreciable difference in the numbers of eggs laid in the traps baited by old and new copra meal, although that conditioned by *Ptinus* attack might have been expected to be unattractive. But, as indicated in the previous paragraph, the speed of larval development in the old meal is inferior to that in the new meal. It is hoped to deal more fully with this problem in a later paper.

Cotton seed meal with 38 per cent. of the eggs laid in traps was more attractive than fishmeal with 18 per cent. Wheatfeed received 16 per cent. and wholemeal flour only 5 per cent. The inner site was equally as good as the outer site, probably because a centre of population had developed in the nearby experimental bags. The adults feeding and laying at the outer site hide during daylight in the cracks at the foot of the nearby wall.

Adults were found to collect under the newspaper of the traps. Adult counts were made weekly, *i.e.*, at the same time as foods were changed and also on the intermediate weeks. Each trap was lifted and the adults clinging to the paper brushed into a petri dish while those on the floor were counted. The disturbed adults dispersed but were not allowed to dive under another trap. The new foods were thus laid on a clean floor and hence new adults were attracted to the new trap foods. Nine counts were made of the number collecting at the two sites, on 21st and 28th August, 5th, 12th, 19th and 25th September, 1st, 8th and 10th October. The corresponding five trap periods for oviposition are as in Table I starting 14th to 28th August. Table II gives the total numbers of adults found associated with each food on these dates together with the numbers of eggs laid in the traps over the same period. If it is assumed that adults hiding under a trap have had the opportunity of feeding and ovipositing in it also, the ratio of eggs to adults shows a marked preference for cotton seed meal and dislike of flour as an oviposition site.

TABLE II.

Eggs found in oviposition traps and number of adults found under traps over the period, 14th August to 10th October, 1946.

	Outer site			Inner site		
	Adults	Eggs	Eggs/ Adults	Adults	Eggs	Eggs/ Adults
Cotton seed meal	654	1,612	2·46	1,097	1,651	1·50
Fishmeal	456	637	1·40	912	474	0·52
Whole flour	529	143	0·27	628	178	0·28
Wheatfeed	387	572	1·48	669	654	0·98
Used copra meal	250	396	1·58	592	776	1·31
Fresh copra meal	274	470	1·71	420	485	1·15
	2,550	3,830		4,318	4,218	

The number of adults given in Table II represents a mean of 480 adults per count at the inner site and, the eggs being laid over 58 days, a mean of 70 eggs per day. On the assumption that most of the adults laying at this site take refuge under these traps at dawn, this gives an estimated oviposition rate of one egg per seven adults per day at this time of year (Aug.–Oct.). This is much below the laboratory figure of 0·8 eggs per adult per day for six months at comparable temperatures given adequate food and water. The limiting factor in warehouses is very likely to be drinking water, for whereas, in laboratory experiments, groups of *Ptinus* adults given drinking water continue to oviposit for long periods, others originally laying as many as seven eggs per adult daily, cease laying altogether in less than three weeks even at high humidity (80 per cent. R.H.), if deprived of drinking water.

Results in an Unheated Building at the Laboratory.

When the warehouse was vacated in October, 1946, 25 of the experimental bags, complete with their insect population, were brought back to the laboratory and placed in an open metal bin fitted with an internal wooden floor six feet square. The bin, the rim of which was coated with sticky banding to prevent the insects from escaping, was situated in an unheated outbuilding.

On 1st January, 1947, an oviposition trap baited with fishmeal was placed on the wooden floor of the bin near the bags. The fishmeal was taken up and renewed at irregular intervals until 27th May and after that it was renewed every weekday. For the period 1st July to 18th September a second trap baited with copra meal was used and from 1st October to 30th November another trap was baited with groundnut meal. The fishmeal trap was continued until 24th March, 1948. As before, trap foods were subsequently kept at 25°C. and 70 per cent. R.H. and the larvae produced were counted a fortnight later. Continuous thermohygrograph records were kept of conditions near floor level in the bin.

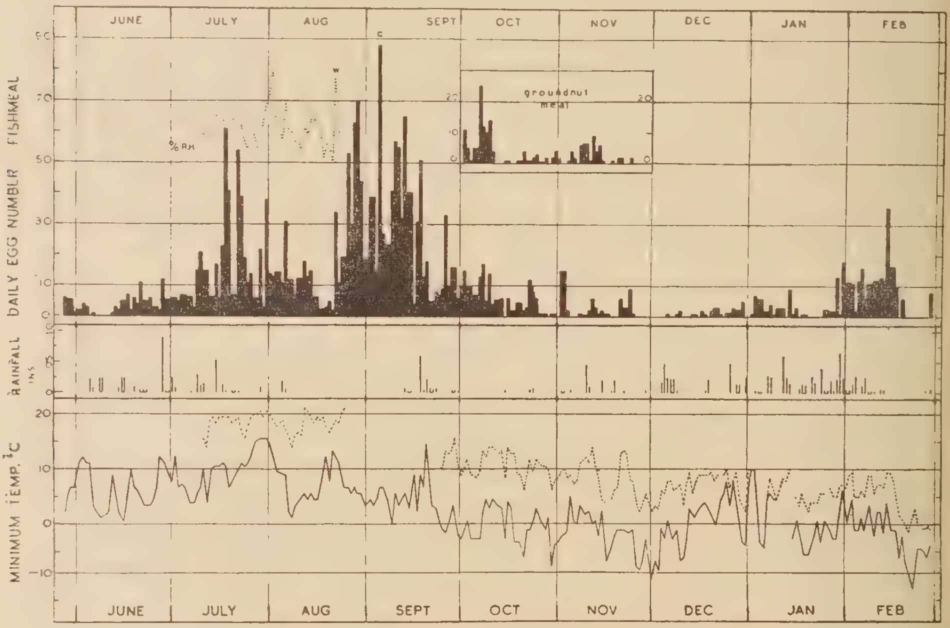

Fig. 1.—*Top*.—Histograms of numbers of eggs laid daily in a bin in traps baited with fishmeal and groundnut meal. The maximum daily relative humidity in the bin is shown by dotted line............

Middle.—Daily rainfall in inches. Adjacent records are joined at base when exact distribution of rain between the days is not known.

Bottom.—Minimum daily grass temperatures———— and minimum daily temperatures in bin............

Fig. 1 shows the numbers of larvae obtained in the fishmeal trap from 27th May onwards and those obtained from groundnut meal later. Fig. 1 also includes the daily minimum bin temperature over the period after late September and shows that oviposition is dependent on temperature at this time of year, there being fair correspondence between peaks and troughs in the two curves. A section of the minimum bin temperature record for July and August is also figured and again there is some correspondence with oviposition rate but the correlation between egg laying and maximum relative humidity is more obvious at this season.

On 22nd August some water was accidentally upset into the bin. The sudden provision of ample drinking water is reflected in a marked increase of oviposition for over three weeks at a time when temperature and humidity were comparatively unfavourable, with minimum outdoor temperatures consistently below 10°C. and thirty-seven rainless days. The first rainy day and the first heavy rain after this dry spell both stimulated an increase in oviposition.

On 5th September the bin was covered all day by a sheet of balloon fabric. As a result, 88 eggs were laid in the fishmeal and 18 in the copra meal during that day and since temperature and humidity were not affected, the increase is presumably due to continuous activity in the dark all day (see C in fig. 1).

Fig. 1 also includes the minimum grass temperature and daily rainfall at the laboratory. These are often the only figures available but from them it is often possible to infer roughly the conditions inside unheated buildings.

In 1947 there were periods of cold which prevented oviposition. One very long such period lasted from late January to late March. A number of short spells are shown in fig. 1 including one in early June. Low humidity did not prevent oviposition completely at any time.

TABLE III.

Monthly totals of eggs laid in oviposition traps baited with fishmeal, copra meal (c) and groundnut meal (g).

Month	Eggs laid
January, 1947	30
February	0
March	19
April	196
May	197
June	105
July	500 (c 53)
August	629 (c 33)
September	817 (c 185 in 18 days)
October	185 (g 115)
November	72 (g 56)
December	34
January, 1948	94
February	234
March (to 24th)	154

The monthly egg totals for the oviposition traps are given in Table III. Groundnut meal was little inferior to fishmeal but copra meal was not laid in to any extent until the water was upset into the bin. In the forty days preceding the water upset, only one egg was laid in the copra meal, another was laid five days later but after another two days it was used considerably though still much less than fishmeal.

Factors affecting Oviposition in Traps.

Ptinus tectus is normally active at dusk and through the hours of darkness; consequently the temperature and humidity at these times would be expected to influence the rate of egg laying. The results obtained in this work substantiate this opinion and confirm that the limiting factors are temperature in winter and water in summer.

The minimum temperature, normally occurring at two or three in the morning, and maximum relative humidity, normally occurring at dawn or soon after have been related to oviposition in fig. 1, but obviously a figure representing a summation of conditions (see Pradhan, 1946) during darkness would give a truer relationship. The lowest temperature at which Ewer and Ewer (1942) obtained eggs from *P. tectus* was 7°C. but on three days, eggs were laid in the bin when the maximum temperature was below 7°C. It seems probable that in the field some laying may occur at 5°C.

The presence of free water for drinking probably has a greater effect on egg laying than relative humidity. The warehouse used in this experiment leaked badly and so there were many wet places during and after rain. The bin at the laboratory was situated near a window which was generally open during the day and consequently the bin was liable to be splashed a little during rain.

Subject to temperature and humidity being favourable, light exerts a great influence on oviposition. The majority of eggs are laid in dim light or darkness and it is certain that the darkness of many British warehouses is a major cause of the quick build up of *Ptinus* populations.

The foods used in the oviposition traps influence the numbers of eggs laid in them when a choice is given. Thus flour attracted few eggs, fishmeal and cotton seed meal many. Three laboratory experiments were made in which *Ptinus* adults were offered the choice of flour, fishmeal, copra meal and wheatfeed. The field results were confirmed in two of these, many eggs being laid in fishmeal and few in flour or copra but, in the third, fishmeal was the least popular food and flour and wheatfeed the most used for egg laying. In a laboratory experiment in which no choice of foodstuff was offered fishmeal and copra meal were laid in much more readily than flour or wheatfeed. Thus it appears that fishmeal is a much better bait than flour for use in a trap designed to measure changes in egg laying rates.

The position of the traps also affects their success. They will receive more eggs the nearer they are to those places in which the insects are hiding. They are best placed near the wall of a store or close to the edge of the stack of stored goods.

Summary.

An account is given of an attempt to estimate changes in the rate of egg laying in a warehouse and in an unheated outbuilding at the laboratory. Small samples of food were placed on pieces of newspaper on the floor, and were renewed regularly, weekly or fortnightly in the warehouse and daily at the laboratory. Larvae were bred from these food samples and counted.

The rate of oviposition was found to depend on temperature, especially in winter, and on relative humidity, especially in summer. It was seldom cold enough indoors, in unheated premises, to prevent oviposition entirely for more than a few days at a time, 5°C. being the approximate minimum temperature for oviposition. It was never so dry at the laboratory that egg laying stopped but this did happen at the warehouse in July, 1946. Free drinking water is necessary for continued oviposition and this was available from rain in both buildings.

Darkness causes an increase in activity and in oviposition and most of the eggs in the foods supplied were laid at night. Foods placed near the daytime hiding places of adults were most used for oviposition.

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THE CONTROL OF THE BLACK SAGE IN MAURITIUS BY *SCHEMATIZA CORDIAE* BARB. (COL., GALERUCID.).

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(Plates XIV and XV.)

The shrub, *Cordia macrostachya* (Jacq.) R. & S., commonly called the Black Sage or " Herbe Condé," is a member of the Boraginaceae which is indigenous to the West Indies, Central America, and Northern South America (Johnston, 1936). It was accidentally introduced into Mauritius about 1890, probably from British Guiana, and subsequently became a serious weed, especially of grasslands and of aloe (*Furcraea gigantea* Vent.) growing areas (Pl. XIV, figs. 1–4 ; Pl. XIV, fig. 5). Wiehe (1946), after investigating the ecology of *C. macrostachya* in Trinidad and in Mauritius, concluded that the invading power of the plant in the latter country was due to a profuse production of viable fruits, and that this in turn was due to the plant's comparative freedom from injurious insects. Reference should be made to this important paper for details of the introduction of *Cordia* into Mauritius, and for an account of its dispersion, ecology, and economic significance in the island.

The idea of controlling this weed by biological means originated in 1939 (files of the Department of Agriculture) but the preliminary work, which concluded with Wiehe's study, could not begin until Kirby and Adamson (unpublished report, 1944) began to tabulate the insects which feed upon *Cordia* in Trinidad. These included two leaf-eating beetles, *Physonota alutacea* Boheman (Cassidid.) and *Schematiza cordiae* Barber (Galerucid.). The introduction of the former insect into Mauritius resulted in failure (Simmonds, 1949 ; Williams, 1950a). The latter, which was originally thought to be *S. lateralis* Jac., and later shown to be a distinct species previously undescribed (Barber, 1947), has effected remarkable control of *Cordia* since its release in 1948. Other promising West Indian species have also been under investigation with a view to their introduction, and in 1950 colonies of a seed-infesting Chalcidoid, *Eurytoma* sp. nr. *howardii* D.T., had been established. This paper, however, describes only the introduction of *Schematiza cordiae,* its bionomics, and controlling effect upon *Cordia* between 1947 and 1950.

Reception and Feeding Tests.

Between June and September 1947, 13 air consignments of *Schematiza* were dispatched from Trinidad by Dr. F. J. Simmonds of the Commonwealth Bureau of Biological Control. They were sent as fully grown larvae and prepupae free of their natural parasites. Mortality in transit was high, and, of just over 5,000 insects sent, only about 500 were received alive as adults. Breeding was commenced under strict quarantine while feeding tests to determine the degree of specificity to *C. macrostachya* were initiated. Most of these tests were carried out in petri dishes containing leaves of the various plants listed below. All three larval instars as well as adults were used, the former being starved for 24 hours and the latter for 48 hours before being placed in the dishes. The mean minimum and maximum daily temperatures in the insectarium during the tests were 18·7° and 26·5°C., respectively. No evaluation of the humidity within the dishes was made.

The following plants were used in tests to determine the specificity of *Schematiza* to its food.

BORAGINACEAE
†_Cordia myxa_ L.
†_C. abyssinica_ K.Sch.
†_Ehretia acuminata_ R.Br.
Heliotropium indicum L.

CONVOLVULACEAE
Ipomoea batatas L.

SOLANACEAE
Nicotiana tabacum L.
Lycopersicum esculentum Mill.
Solanum tuberosum L.
S. melongena L.
S. auriculatum Ait.
Capsicum annuum L.

COMPOSITAE
*_Lactuca sativa_ L.
Dahlia variabilis Desf.
Eupatorium pallescens DC.

COMMELINACEAE
Wikstroemia indica Mey.

BIGNONIACEAE
Tabebuia pallida Miers

VERBENACEAE
Lantana camara L.
Stachytarpheta jamaicensis Vahl.

GRAMINEAE
Saccharum officinarum L.
Zea mays L.
Stenotaphrum dimidiatum Brongn.

MUSACEAE
Musa cavendishii Lamb.

SAPINDACEAE
Euphoria longana Lam.
Litchi chinensis Sonn.

MALVACEAE
Gossypium sp.

OLEACEAE
Ligustrum walkeri Decne.

UMBELLIFERAE
Daucus carota L.
Petroselinum sativum Hoffm.

CUCURBITACEAE
Cucurbita maxima Duchesne
*_Sechium edule_ Sw.

CRUCIFERAE
†_Brassica oleracea_ L.
*_B. oleracea_ var. _botrytis_ Mill.
B. chinensis L.
*_B. alboglabra_ Bailey

LILIACEAE
Allium cepa L.
Furcraea gigantea Vent.

LEGUMINOSAE
*_Phaseolus vulgaris_ L.
Arachis hypogaea L.
Tamarindus indica L.
Leucaena glauca Benth.
*_Delonix regia_ Rafin.
Caesalpinia sepiaria Roxb.
Cajanus cajan Millsp.
Vigna catjang Walp.
Pisum sativum L.
Albizzia lebbek Benth.
Haematoxylon campechianum L.
Cassia siamea Lam.
Canavalia ensiformis DC.
Crotalaria sp.

ANONACEAE
Annona sp.

LAURACEAE
Persea americana Mill.
Cinnamomum camphora Nees
Litsea glutinosa C. B. Rob.

MORINACEAE
Artocarpus integrifolia L.
A. incisa L.

ROSACEAE
Prunus persica Stokes
Fragaria vesca L.
Rubus roridus Lindl.
Rosa sp.

BROMELIACEAE
Ananas comosus Merr.

LYTHRACEAE
Lagerstroemia flos-regina Retz.

MYRTACEAE
Psidium guajava L.
P. cattleianum Sab.
*_Eugenia cumini_ Merr.
E. jambos L.
Eucalyptus spp.

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*Indicates nibbling or puncturing of the leaves.
†Indicates appreciable leaf feeding at some stage.

Rubiaceae
Coffea sp.
Vangueria madagascariensis Gmel.
Eriobotrya japonica Lindl.

Moraceae
Ficus sp.

Rutaceae
Citrus aurantifolia Swingle
C. sinensis Osbeck

Caricaceae
Carica papaya L.

Geraniaceae
Pelargonium sp.

Dioscoreaceae
**Dioscorea bulbifera* L.

Palmae
Cocos nucifer L.

Euphorbiaceae
Manihot utilissima Pohl.
Acalypha grandis Benth.

Anacardiaceae
Mangifera indica L.

Combretaceae
**Terminalia arjuna* W. & A.
T. catappa L.

Theaceae
Thea sinensis L.

Chenapodiaceae
**Beta vulgaris* L.

Celastraceae
Elaeodendron orientale Jacq.

Casuarinaceae
Casuarina equisetifolia L.

Under the above conditions, larvae and adults fed to varying degrees on several plants, but notably on *Cordia myxa*, *C. abyssinica*, and *Ehretia acuminata*, all of the Boraginaceae, and also on cabbage (*Brassica oleracea*). The life-cycle could not, however, be completed on any of the test plants, although it occurred readily on leaves of the control, *C. macrostachya*, under the same conditions. On cabbage, the second- and third-larval instars fed fairly readily, and some of the latter attained the adult stage (Moutia, 1948). The adults would not feed or oviposit on cabbage leaves. No *appreciable* feeding occurred with any of the other plants ; at the most the leaves were punctured or nibbled, and death followed within a few days.

Further tests were made with young potted cabbage plants in cages. It was found that larvae would not feed at all under such conditions, but repeatedly wandered off the plants until death ensued. More tests were not made with the Boraginaceous plants on which feeding occurred, for this family has no member of economic value in Mauritius.

These results were almost identical with those of similar tests conducted in Trinidad (Simmonds, 1950) and it was concluded that *Schematiza* could be released with little risk of danger to plants unrelated to *C. macrostachya*.

Breeding and Liberation.

Liberation of laboratory bred insects commenced in March, 1948 (Williams, 1949).

Oviposition was obtained in cages containing *Cordia* shoots in water. Fresh shoots were substituted at intervals of two to three days, and the interiors of the cages were sprayed daily with clean water. The egg batches were cut away with a small portion of the leaf to which they adhered and placed between freshly picked leaves in a closed jar ; this maintained a high humidity which was favourable to hatching. Newly emerged larvae were transferred to fresh leaves in closed jars having a layer of plaster of paris in the bottom to absorb excess moisture. When more food material became necessary, the larvae were removed to newly picked leaves and replaced in the jars. They would wander off the leaves when fully grown and they were then placed in damp moss to pupate.

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*Indicates nibbling or puncturing of the leaves.
†Indicates appreciable leaf feeding at some stage.

When breeding was terminated in February, 1949, approximately 18,000 laboratory reared adults had been released in 18 localities over the island. The number released in each locality varied between 250 and 2,000, and the localities were selected so as to cover as wide an environmental range as possible.

The breeding was stopped because very large populations had developed at many of the liberation sites, and further distribution was carried out with adults collected from those where they were particularly numerous. About 60,000 were so collected and released between February and April, 1949 (Williams, 1950b).

The Biology and Bionomics of *Schematiza*.

Simmonds (1950) has given a full account of the biology of *Schematiza* in the West Indies, including descriptions of the various stages. Only the essentials, with the bionomic data obtained in Mauritius, will therefore be given here.

The eggs are laid in irregular batches of about 30 on either side of *Cordia* leaves, but usually on the under side. The larvae tend to be gregarious, especially when young, and they gnaw away the surface tissues of the leaves sometimes perforating them when nearing maturity. Skeletonised areas result, and if these are large the leaves dry out and fall. There are three larval instars which are readily distinguishable by the width of the head capsule. The following figures were derived from measurements of 50 larvae of each instar.

	Mean head width (mm.)	S.D.
1st instar	0·321	0·005
2nd instar	0·498	0·003
3rd instar	0·744	0·037

Pupation occurs in the debris of vegetation beneath *Cordia* bushes. The duration of the life-cycle from egg to adult varies from 20 to 50 days in Mauritius according to seasonal and climatic changes.

The adults usually perforate the leaf blade when feeding (Pl. XV, fig. 5), and severe holing cause the leaves to wither and then fall. Mating occurs within a week of emergence and the first eggs are laid a few days later. In the laboratory the adults usually lived between three to eight weeks, while the females oviposited fairly regularly each day throughout the greater part of their lives. Mating occurred at frequent intervals, but females allowed to mate only once produced as many fertile eggs as those which mated several times. In the summer, the total number laid by a female averaged 750, while in winter it was approximately half this number.

One characteristic that has not been recorded from the West Indies is that of feeding upon inflorescences. In Mauritius, both larval and adult stages sometimes feed upon the unripe fruits and the soft outer tissues of inflorescence stalks, even when foliage is in good supply, but such feeding is more pronounced when foliage is scarce. If the endocarp of an attacked fruit has not hardened, the seed is destroyed, while the nibbling of the inflorescence stalk causes others to fall prematurely (Pl. XV, fig. 4). Newly hatched larvae were able to complete their development in the laboratory when fed only with inflorescences, but mortality was high and the resulting adults were abnormally small.

A dormant stage occurs in the field during the winter, the nature of which varies with the climate of the various regions of the island. The winter, or cool season from May to October, is comparatively dry while the summer, or hot season, which lasts from November to April, is accompanied by heavy showers with a maximum precipitation in March. It may be said that there are two fairly distinct climatic regions, the uplands and the coastal belt, the latter widening in the north into an extensive plain. The mean annual temperature in the uplands varies from 22·0°C. to 23·3°C., and the annual rainfall from 100 ins. to 175 ins., while in the coastal regions the figures are 25·5°C. to 27·0°C. and 25 ins. to 75 ins., respectively.

The dormant period of *Schematiza* lasts roughly from June to December, and in most regions it consists of a slowing down of general activity. Few eggs are laid and development is prolonged, while the adults, although they continue to feed to some extent, are much less in evidence and probably spend most of their time resting off their food-plant. The dormancy is more pronounced in the drier climates of lower altitudes, and in some coastal areas which are subject to severe drought in winter, quiescence is probably complete, for all stages are apparently absent from the bushes at this time. There is thus a gradation in the degree of dormancy according to altitude and its incumbent climatic variations. The time of emergence from the dormancy also varies, occurring in December in the uplands, and several weeks later in coastal districts. Also, in the former regions reactivity is more gradually apparent than in the latter.

The seasonal and climatic differences which regulate the activity of *Schematiza* have a parallel effect upon *Cordia*, for it loses its summer foliage in regions which are very dry in winter (Pl. XIV, figs. 3 and 4), while in sheltered upland areas seasonal foliage differs only in the degree of profuseness. The activity of *Schematiza* in different regions throughout the year is thus closely synchronised with that of its food-plant ; the severest injury is inflicted when plant growth is active, but in upland regions where growth is retarded during the winter, the activity of the insect is correspondingly reduced.

Progress by Periods.

The year 1948 was one of dispersion and colonisation with laboratory reared insects. The injury to *Cordia* was negligible, for liberations commenced in March which is towards the close of the hot season, and the released insects passed into the dormant stage. Consequently, few insects were visible at the majority of colonisation centres until December, and noticeable defoliation was restricted to within about 100 yards of the points of release.

Rapid multiplication became evident towards the end of the year as the hot wet season began, and by February and March, 1949, most colonies had given rise to enormous populations. During the brief period before these began to scatter, phenomenal numbers of adults were seen stripping bushes of leaves, inflorescences, and even the softer bark as the colonies expanded from their centres. Around the periphery of these colonies, 15,000 to 20,000 beetles per bush were often estimated, but this lasted for only a short time as natural dispersion followed rapidly and was almost island-wide by the later months of the year. As already described, this dispersion was assisted by distributing field collected colonies. Severe injury to *Cordia* bushes was almost equally widespread owing to the great numbers of the insect which developed before the dormant stage set in. The adult population was then so large that considerable injury to bushes continued through much of the ensuing period of relative inactivity. At the end of 1949 extensive areas of scrub, particularly in the north and east of the island, had been denuded of leaves, flowers, and fruits, and much had been so for about six months.

Schematiza was less numerous in 1950, apparently on account of a food shortage in the regions where it had been excessively abundant in the previous year and the adults, emerging from dormancy, found insufficient food for their needs. In the south and some south-westerly areas where *Cordia* had not suffered so severely, there was however a repetition of the activity of the previous year on a somewhat smaller scale. Despite the smaller numbers in most regions, new growth, which denuded scrub began to produce after the summer rains, was everywhere suppressed, while the attack of bushes which had escaped severe injury progressed steadily. When dormancy again ensued it could be said that *Schematiza* had either denuded, or defoliated usually to a severe degree, nearly all dense growth of *Cordia*.

More scattered growth such as occurs in waste plots, along roadsides, etc., has not, as a rule, suffered so severely. Defoliation is often patchy, and bushes may be seen flourishing quite near to some which have been severely attacked.

Nature of the Control effected by *Schematiza*.

Cordia bushes are very resistant to mechanical injury and uprooting is normally necessary to kill them, but those which have been denuded by *Schematiza* (Pl. XIV, fig. 6 ; Pl. XV, figs. 1 and 2) begin to die back from the tips of their branches. If new foliage is continually prevented from developing, the die-back continues, more slowly with the increasing girth of the branches, until death of the plant eventually results. Bushes about four feet high may still have the basal portions of their branches alive after 12 months without foliage, but smaller plants usually die within this period. *Schematiza* has killed very many bushes in this manner throughout the island. The dead, and the nearly dead scrub is often exploited as firewood, and consequently much *Cordia* has completely disappeared.

Following a prolonged absence of foliage, other members of the vegetation which could not previously compete with *Cordia*, grow up quickly. The first invaders are often climbing plants, such as *Passiflora suberosa* and *Ipomoea* sp., which spread in masses over the stripped bushes often bearing them to the ground (Pl. XV, fig. 3) ; *Lantana camara* is also in the fore, and with its long stems and thick foliage completely envelops the bare *Cordia* branches. The vegetation which was previously dominated thus contributes to the final subjugation of *Cordia*. Cutting back of scrub is now usually effective, for with the presence of *Schematiza* the bushes cannot regain their dominant status.

When Wiehe (1946) determined that the aggressiveness of *Cordia* was due to its profuse production of viable fruits, he estimated that one acre of scrub was producing about 134 million annually. The stripping of scrub by *Schematiza* and the death of much of it has drastically reduced this seed production. Less severe injury must also be taken into account ; Callan (1948) and Simmonds (1951) have shown that partial defoliation reduces reproduction, while as already described, associated with this foliar effect is the destruction of fruits before they reach maturity by direct attack upon the inflorescences.

Food-Plant Specificity in the Field.

The conclusions drawn from the results of the feeding tests prior to the release of *Schematiza* have been verified by subsequent events, for the life-cycle has not been observed to occur on any plant other than *C. macrostachya*, despite self-created food shortages of a most acute nature. Reports of injury to cultivated plants have always proved to be false, most of these having arisen by inference when *Schematiza* had been seen resting on foliage suffering from insect injury of various kinds. The abundance of the beetles naturally favours such reports.

Although specific to *C. macrostachya* for development, the adults will feed and oviposit upon two related plants, *C. abyssinica* and *C. myxa*. The injury sustained by the former is chiefly confined to seedlings and very young plants which may suffer complete defoliation if *Schematiza* is numerous in their vicinity. The leaves of grown trees merely have small patches of the surface tissues gnawed away. Young plants of *C. myxa* have not been seen, but with grown trees the behaviour of *Schematiza* is similar. Egg batches are frequently laid on both plants, but the larvae disappear shortly after emergence, having made only small abrasions of the leaf around the egg shells. It is presumed that they die.

Schematiza is not attracted to *Ehretia acuminata*, the other Boraginaceous plant used in the feeding tests. This applies also to two other species of *Cordia* present in Mauritius, *C. alliodora* Cham. and *C. holstii* Gurke ; the former has been experimentally planted on a small scale as a timber tree, while the latter is grown as a specimen in the Royal Botanic Gardens.

Parasites and Predators.

Frequent collections of eggs and larvae show that no Mauritian parasitic insects have adapted themselves to *Schematiza* as a host.

The only predator which has been observed is *Afrius williamsi*, a Pentatomid not recorded prior to this work and which has recently been described by Miller (1951). The first nymphal instar of *Afrius* feeds only upon plant juices, but all later instars, including the adult, are obligate predators although the plant feeding habit persists. Both larval and adult *Schematiza* are attacked, but the latter infrequently and usually soon after emergence when the integument is soft. In the laboratory it is polyphagous, but although the same is undoubtedly true in the field, it has been observed on *Cordia* bushes preying upon *Schematiza* only, which it does in all regions of the island. It is frequently numerous and it evidently aggregates near a good supply of suitable prey. *Afrius* cannot be regarded as an adverse factor of practical significance, as evinced by the results which have been obtained, in spite of its presence.

There are probably other predators, the wasp, *Polistes hebraeus* (F.), the Mantid, *Polyspilota pustulata* (Stoll), and also birds are suspected, but any effect they may have is of little concern.

Discussion.

Schematiza cordiae is widely distributed in Trinidad but causes little injury to *Cordia*, never apparently destroying entire leaves (Simmonds, 1948). This author has shown it to be heavily parasitised and has illustrated the rigorous control exerted by these parasites. In Mauritius where there are no serious adverse faunistic elements, the high fecundicity and wide climatic range of the insect have resulted in the extensive destruction of *Cordia* and checked the reproduction of surviving bushes. The multiplication of *Schematiza* was so rapid that ascendancy over the plant was achieved after the passage of one complete summer of unchecked activity. There has since been a decrease in the intensity of its activity which will undoubtedly continue until an equilibrium is established, when the injury inflicted to *Cordia* will be erratic and perhaps confined to partial defoliation and control of seed production. Good control of *Cordia* is now assured, but it is intended to pursue for some time the investigation of other possibly beneficial species to contribute, if possible, to the future reduced status of the plant.

Summary.

Cordia macrostachya (Jacq.) R. & S., is a Boraginaceous shrub indigenous to the West Indies, Central America, and northern South America. It was accidentally introduced into Mauritius about 1890, probably from British Guiana, and became a weed of major importance.

A comparative ecological study of *Cordia* in Mauritius and in Trinidad showed that its invading power in the former island rested upon a profuse production of viable fruits, whereas in Trinidad, where the plant is of no agricultural concern, fruit production was controlled by the combined action of a number of insects. It was accordingly decided to introduce suitable species of these insects into Mauritius.

The first to be introduced was a leaf-eating Cassidid beetle, *Physonota alutacea*, but as described elsewhere, this introduction, which took place in 1947–48, was not a success.

The introduction of the second species, *Schematiza cordiae* Barber, and its result, is described in the present paper. This Galerucid is also a leaf-feeder, but it will sometimes feed upon the inflorescences.

Through the agency of the Commonwealth Bureau of Biological Control, air consignments of *Schematiza* were received between June and September, 1947. Breeding was started, and after feeding tests in both Mauritius and Trinidad had established its specificity to *C. macrostachya*, the release of laboratory reared adults was commenced in March, 1948. In February, 1949, after about 18,000 had been released, breeding was no longer necessary for large populations had developed in the field and were inflicting severe injury to *Cordia* scrub. Dispersion of the insect over the island followed quickly, and was assisted by the collection and distribution of about 60,000 adults. *Schematiza* became extremely abundant so that by the middle of 1950 most expanses of scrub in the island had either been stripped of leaves and inflorescences, or had generally been severely defoliated.

As a result, much scrub is dead owing to a prolonged period without foliage, much is being subjugated by other vegetation, while partial defoliation and direct inflorescence attack have reduced the reproduction of other bushes.

This rapid control of *C. macrostachya* is attributed to the high fecundity of the *Schematiza* combined with its freedom from parasites and important predators, and to its suitability to the climates of all *Cordia* infested regions.

Acknowledgements.

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The contributors to this successful phase of the work are acknowledged in the text, but in addition credit is due to all members of the Entomological staff of the Department of Agriculture.

The writer wishes to acknowledge in particular Mr. A. L. Moutia for performing most of the feeding tests, and also the acting Director of Agriculture, Mr. N. Craig, for his constant support and many helpful suggestions.

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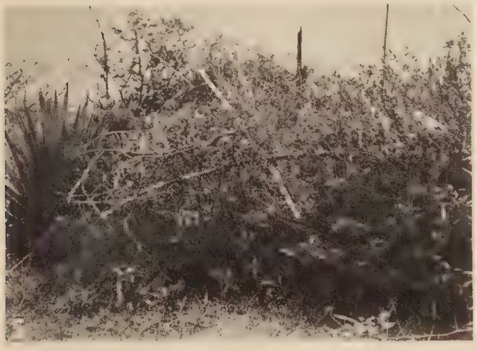
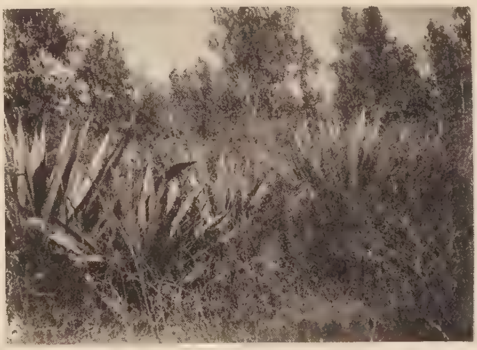




Views of *Cordia* growth in 1948.

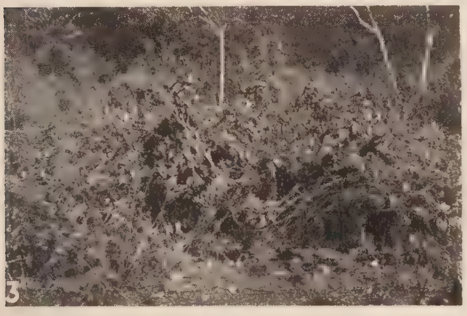
FIG. 1. Typical dense scrub of lowland regions.

FIG. 2. Growth at about 1,100 feet, at which altitude privet, brambles, etc., begin to displace *Cordia*.

FIGS. 3, 4. Summer and winter growth in a lowland district.

FIG. 5. A view of *Cordia* growing among aloe plants (*Furcraea gigantea*).

FIG. 6. *Cordia* among aloes after attack by *Schematiza*.



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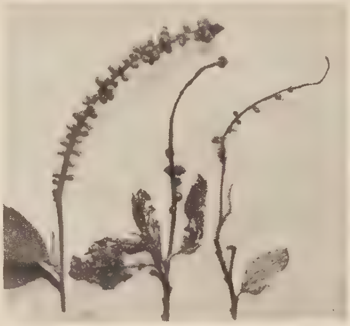

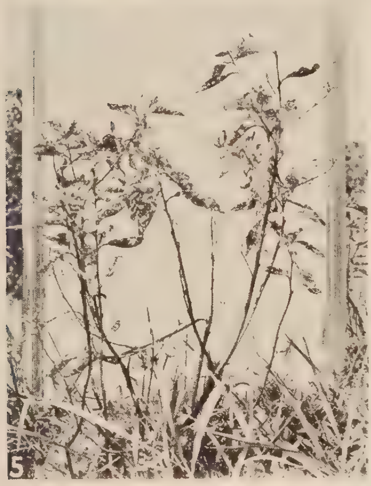

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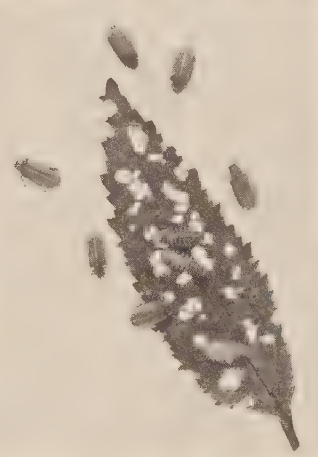

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FIGS. 1, 2. *Cordia* bushes after attack by *Schematiza.*

FIG. 3. Attacked bushes pressed to the ground by masses of climbing plants.

FIG. 4. Healthy and attacked *Cordia* inflorescences.

FIG. 5. A *Cordia* bush under attack by *Schematiza,* the lower leaves have fallen and the remainder, which show the characteristic holing of the adult insect, are drying.

FIG. 6. Mature larvae and adults of *Schematiza.*

FOUR NEW SPECIES REPRESENTING TWO NEW GENERA OF BRYOCORINAE ASSOCIATED WITH CACAO IN NEW BRITAIN (HEMIPTERA, MIRIDAE).

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Thanks to the courtesy of Mr. G. S. Dun, Government Entomologist to the Territory of Papua and New Guinea, we have been able to study three interesting new species of Mirids found damaging cacao plants in New Britain. The first was discovered by Mr. Dun early in 1950 in abundance at Kabeira Plantation about six miles from the Lowland Experimental Station at Keravat where considerable damage had been done to the crop. This species was at first thought to be the West African *Bryocoropsis laticollis* Schumacher, 1917, which might have been accidentally introduced into New Britain. Closer investigation showed that it belonged to a distinct genus and species (*Parabryocoropsis typicus*, gen. et sp. n.). In September 1950, Mr. Dun found a second species attacking an isolated block of Criollo cacao at the Keravat Experimental Station. On examination this proved to belong to still another genus and species (*Pseudodoniella pacifica* gen. et sp. n.).

A search in the British Museum (Natural History) collections disclosed a third species belonging to the first genus (*Parabryocoropsis cheesmanae*, sp. n.), collected by Miss Cheesman at Kokoda in Papua. It was then discovered that amongst the material sent by Mr. Dun from the Criollo cacao was a fourth species (*Parabryocoropsis duni* sp. n.).

The following are the descriptions of the new genera and species.

Pseudodoniella, gen. n.

Bryocorinae, characterised by the shape of the body, hemielytra partially covering the connexivum, large size of inflated scutellum and sexual differences in the embolium.

General shape elongate, with pronotum strongly declivous in front, the scutellum very prominent.

Head twice as broad as long, slightly exserted with a short neck, front provided with a central tubercle which has two apical, blunt points, not reaching the anterior margin of the first antennal segment ; tylus prominent, separated from the front by a line running just below the frontal tubercle, eyes situated at the apex of a short peduncle, equal in length to the width of one eye, posterior margin with a light semicircular emargination, separated from the pronotum by a distance equal to half the width of one eye.

Antennae with segment I very short, as long as wide, provided with a few setae on internal side ; II incrassate toward apex, provided with a few hairs and much more numerous setae, six times as long as the first ; III irregularly incrassate toward apex and IV club shaped, both with hairs and setae.

Pronotum strongly punctured and declivous in front, calli small, horizontal, smooth, brilliant, collar as wide as segment III of antennae at apex, disc convex, the posterior border raised over the hemielytra on the sides but covered on middle, posterior lateral angles laminately reflexed, pilosity short and erect.

Scutellum shield shaped as in fig. 1, very prominent, strongly punctured, the apex blunt, provided with short, erect spine-like bristles.

Hemielytra densely covered with very fine hairs, corium and embolium a little narrowed in the middle (male) or parallel (female), embolium in the male reaching the cuneus and of same width throughout, in the female strongly widened toward apex ; cuneus about twice as long as wide on base, with acute apex and slightly curved inner margin ; membrane smooth, the veins forming an acute angle which reaches beyond the cuneal apex, apical or free area equal in length to that of one membranal cell.

Rostrum reaching the apex of middle coxae ; legs short, covered with fine hairs and short, dark spines more numerous on the tibiae ; tarsal segments of approximately equal length, the third incrassate ; claws of the Bryocorinae type with pseudarolia united to the basal half.

TYPE OF GENUS : *Pseudodoniella pacifica*, n. sp.

This genus has a certain similarity with the West African *Odoniella* Haglund, 1895, the Australian *Volkelius* Distant, 1904 and with the Tibetan genus *Rhopaliceschatus* Reuter 1893, but is readily distinguished by the frontal tubercle and shape of scutellum.*

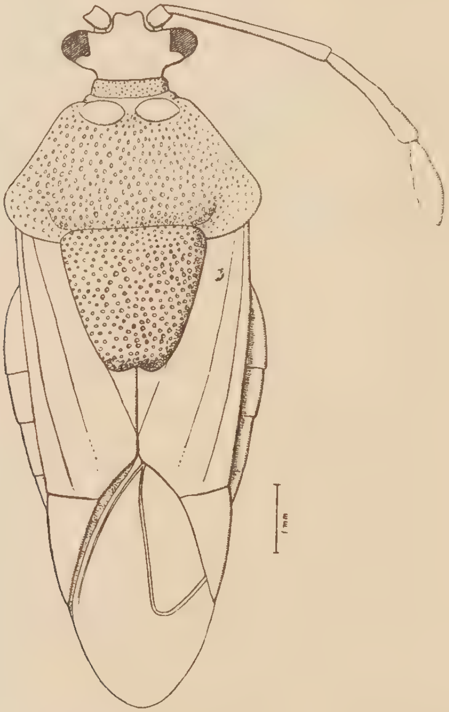

Fig. 1.—*Pseudodoniella pacifica*, gen. et sp. n. ♀.

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*Reuter's figure of *Rhopaliceschatus* (1903, Öfv. Finska Vet.-Soc. Förh., **45** no. 16, Tab. 2 fig. 1) is inaccurate in the structure of the paired anterior tubercles of head and in the failure to show the clypeus which is actually visible from above between the two tubercles. The scutellum is different from that in the other three genera being hemispherical as opposed to shield shaped.

Pseudodoniella pacifica, sp. n. (figs. 1, 2 and 4 *c*, *f*).

Characterised by the colour and male genitalia.

MALE. Length 9·5 mm., width 3·0 mm. *Head* : length 0·7 mm., width 2·0 mm., vertex 1·08 mm. *Antennae* : segment I, length 0·38 mm., width 0·32 mm. ; II, length 2·6 mm. ; III, 1·8 mm. ; IV, 1·2 mm. *Pronotum* : length 1·7 mm., width at base 3·5 mm. *Cuneus* : length 1·3 mm., width at base 0·7 mm. *Rostrum* : length 2·2 mm.

Colour : brick reddish with dark membrane ; antennae infuscate toward the apex, eyes dark chestnut, head and pronotum lighter, almost luteous, apex (female) and apical half and lateral margins of scutellum (male) black, cuneus reddish, membrane dark (except base), veins dark with reddish tinge, tibiae yellow on apical half, sternal region yellow with mesosternum dark, abdomen dark with a greenish tinge on the margins and first segment.

Morphological characters as given for the genus.

Male genitalia : aedeagus (fig. 2) simple, basal plate lyriform, chitinised, with a cup-shaped sclerite at its base, intersegmental (8, 9) foramen opening into a chitinised fig-shaped ejaculatory reservoir from which runs a tracheiform ejaculatory duct widening to form the membranous endosoma. Phallosoma with one side flattened and chitinised.

Left clasper (fig. 4 *c*) falciform with a few small conical tubercles on face and a distinct dorsal seta. Right clasper (fig. 4 *f*) thumb-shaped with a basal collar, dorsal surface of apical third with small conical tubercles.

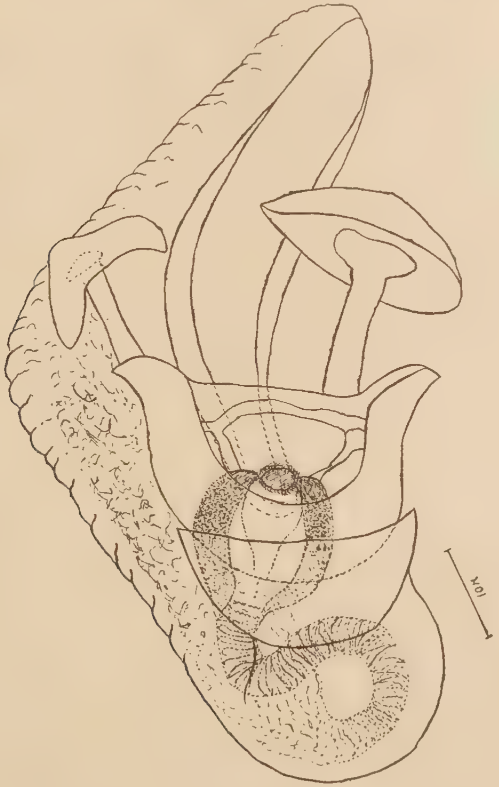

Fig. 2.—*Pseudodoniella pacifica,* gen. et sp. n. Aedeagus.

FEMALE. Slightly longer and wider than male, similar in colour and dimensions of head, antennae and rostrum.

HOST PLANT. Taken on Criollo cacao.

HOLOTYPE. Female, Lowlands Experimental Station, Keravat, New Britain, 7.x.1950 (*G. S. Dun*). British Museum (Nat. Hist.) Collection.

ALLOTYPE. Male, same data as type.

PARATYPES. Two males and one female, same data as type.

Parabryocoropsis, gen. n.

Bryocorinae, characterised by the puncturation and smooth shining tubercular swellings of the pronotum and scutellum, the latter strongly inflated and rounded, pronotum very declivous in front, connexivum not covered by the hemielytra.

General shape stout. Head much wider than long, slightly exserted, with a short neck, vertex with convergent ridges or furrows, the frontal tubercle continued by two lateral blunt points forming a V shape, tylus produced with a median depression, twice longer than the first antennal segment ; eyes situated on short peduncle equal in length to the width of one eye, the posterior orbit with light concavity, separated from the pronotum by a distance equal to half its width.

Antennae with segment I very short, twice as long as wide ; II incrassate toward the apex, strongly and shortly pilose, with fine hairs and setae intermixed, five to six times as long as I, the third irregular, incrassate toward apex, slightly longer than width of vertex, IV thickest at middle, club-shaped.

Pronotum with collar equal in thickness to apex of second antennal segment, calli smooth, shining, separated by a punctured area, pronotum strongly declivous toward the head, coarsely punctured and provided with tubercular, irregular, shining swellings, disc convex, the posterior margin covered in middle by scutellum, raised on the sides where it ends in a laminate edge, the posterior angles rounded.

Scutellum strongly inflated, covering the posterior margin of pronotum, punctured and with the irregular tubercular swellings mentioned for pronotum, covering the clavus with exception only of extreme apex and base, provided with stiff spine-like setae.

Hemielytra covered by delicate and compact pilosity, embolium well marked in both sexes, enlarged at apex ; cuneus with acute apex, about twice as long as wide at base, slightly curved internally ; membrane with vein forming an acute angle.

Rostrum reaching the median coxae ; tibiae with strong hairs, especially the hind ones which are thickened at apex and also provided with very small spines.

TYPE OF GENUS : *Parabryocoropsis typicus*, n. sp.

This genus is closely related to *Distantiella* China, 1944 from West Africa, differing however by the apically rounded scutellum, declivity of pronotum, structure of hind tibiae and antennae with apices of segments not so strongly inflated. It has also strong resemblance with the West African *Bryocoropsis* Schumacher, 1917 from which it is easily differentiated by the presence of the irregular marked tubercular swellings of the pronotum and scutellum, the much shorter frontal tubercles, the processes not longer than wide at base, the lateral margins of pronotum only slightly emarginated before laminate rounded lateral angles, scutellum without a median longitudinal sulcus at base and by the extremely swollen scutellum.

Parabryocoropsis typicus, sp. n. (figs. 3 and 4 *a, d*).

Characterised by its colour, shape of the body, size and male genitalia.

MALE. Length 7·6 mm., width 3·7 mm. *Head* : length 0·7 mm., width 1·6 mm., vertex 1·00 mm. *Antennae* : segment I, length 0·38 mm., width 0·3 mm. ; II, 2·0 mm. ; III, 1·3 mm. ; IV, 1·0 mm. *Pronotum* : length 1·7 mm., width at base 3·5 mm. *Cuneus* : length 1·0 mm., width at base 0·6 mm. *Rostrum* : length 1·7 mm.

Colour : dark testaceous ; head, and antennae lighter, the fourth segment darker than others, calli and scutellum in some specimens with dark, shining tubercles, cuneus brilliant metallic black, apex of cuneus and small membranal area pallid, membrane dark. Underside testaceous, the sternum and abdomen lighter in the middle.

Morphological characters as given for the genus.

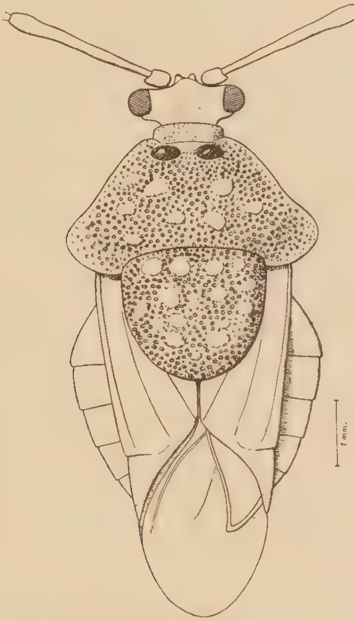

Fig. 3.—*Parabryocoropsis typicus*, gen. et sp. n. ♂.

Genitalia : aedeagus of same type as *Pseudodoniella* but less chitinised. Left clasper (fig. 4 *a*) falciform, with apical fourth bent at right angles to basal three-fourths with a few small conical tubercles on middle of dorsal surface of blade. Right clasper (fig. 4 *d*) finger-like with apex pointed, basal collar similar to that in *Pseudodoniella pacifica* ; a few small conical tubercles on apical fourth.

FEMALE. Similar to male in colour and dimensions, slightly larger and more robust.

HOST PLANT. Cacao.

HOLOTYPE. Male, Kabeira Plantation near Lowlands Experiment Station, Keravat, New Britain, March 1950 (*G. S. Dun*). British Museum (Nat. Hist.) Collection.

ALLOTYPE. Female, same data as type.

PARATYPES. Ten males and two females, same data as type.

This species can be readily distinguished from the following by its colour, size, more strongly inflated scutellum and shorter antennal segments.

Parabryocoropsis cheesmanae, sp. n. (figs. 4 *b*, *e*).

Characterised by its colour, relative size of antennal segments and male genitalia.

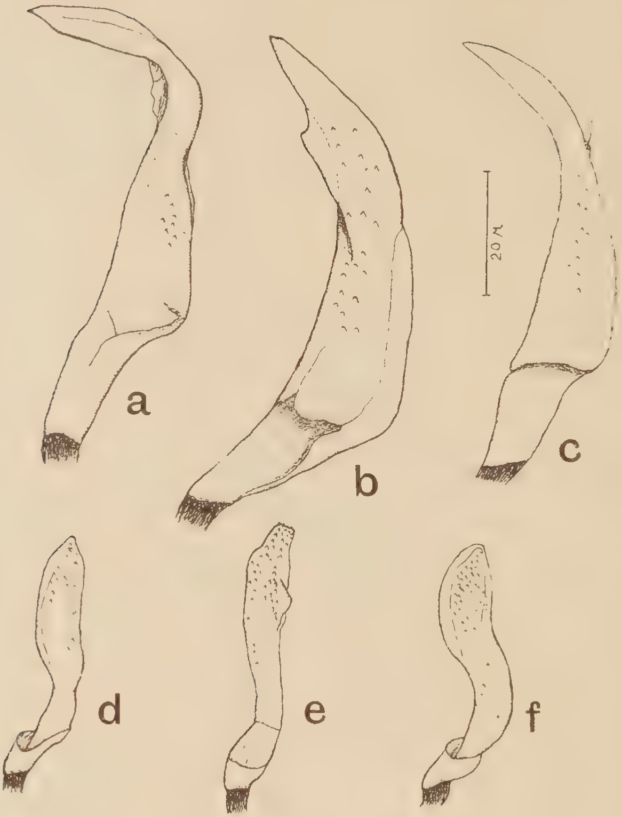

Fig. 4.—*Parabryocoropsis typicus*, gen. et sp. n. ♂. *a*, left clasper ; *d*, right clasper.
Parabryocoropsis cheesmanae, gen. et sp. n. ♂. *b*, left clasper ; *e*, right clasper.
Pseudodoniella pacifica, gen. et sp. n. ♂. *c*, left clasper ; *f*, right clasper.

MALE. Length 8·6 mm., width 3·4 mm. *Head* : length 0·6 mm., width 1·9 mm., vertex 1·08 mm. *Antennae* : segment I, length 0·38 mm., width 0·23 mm. ; II, 2·5 mm. ; III, 1·3 mm. ; IV, mutilated. *Pronotum* : length 1·5 mm., width at base 3·4 mm. *Cuneus* : length 1·2 mm., width at base 0·7 mm. *Rostrum* : length 1·8 mm.

Colour : dark testaceous ; head, collar, calli, posterior margin of pronotum, base of hemielytra and base of cuneus lighter, antennae with segments II, III and IV becoming progressively darker, disc of pronotum dark brown, scutellum black, corium and cuneus darker toward apex, tarsi luteous, claws dark.

Morphological characters as given for genus.

Genitalia : aedeagus normal for the genus. Left clasper (fig. 4 *b*) broadly falciform with a distinct tooth on inner edge of blade, excavated toward base and surface with scattered small conical tubercles. Right clasper (fig. 4 *e*) finger-like with truncated apex and a blunt tooth on inner edge towards apex ; numerous small conical tubercles on apical third.

FEMALE. Similar to male in colour and dimensions, slightly larger and more robust.

Host Plant. Unknown, probably cacao.

Holotype. Male, Papua, Kokoda, 1,200 feet, April 1933 (*L. E. Cheesman*). British Museum (Nat. Hist.) Collection 1933—427.

Allotype. Female, same data as type.

This species is distinguished from *P. typicus* by its colour, larger size, shape of scutellum and male genitalia.

It is dedicated to Miss L. E. Cheesman who has done so much to increase our knowledge of the fauna of the S.W. Pacific.

Parabryocoropsis duni, sp. n.

Characterised by the relative length of the antennal segments, structure of the II, colour uniform with darker membrane and black metallic cuneus.

Female. Length 9·5 mm., width 3·1 mm. *Head*: length 0·7 mm., width 2·0 mm., vertex 1·20 mm. *Antennae*: segment I, length 0·4 mm., width 0·3 mm.; II, 2·2 mm.; III, 2·0 mm.; IV, mutilated. *Pronotum*: length 2·1 mm., width 3·4 mm. *Cuneus*: length 2·4 mm., width at base 0·7 mm.

Colour: brick testaceous with cuneus and membrane dark; antennae gradually darker toward apex, the last two segments black; eyes dark brown, scutellum brilliant infuscate with a brick coloured area in front, cuneus dark, except in extreme base and apex, membrane uniformly black; legs brown with apices of femora and the tibiae with a reddish tinge, tarsi pallid, claws brown, sternum pallid with dark mesosternum, abdomen testaceous with lateral longitudinal luteous band, in the centre of which, on segments III, IV, V and VI, there is an ocellar dark spot. The head, collar, calli and a faint line on internal side of embolium with reddish tinge.

Structure: Second antennal segment with irregular surface, provided with hairs and some stout setae, the third segment with three main swellings; frontal tubercle of head with a few setae; posterior femora curved inwards, the tibiae only slightly longer than the femur.

Male. Unknown.

Host Plant. Criollo cacao.

Holotype. Female, Kabeira Plantation near Lowland Experiment Station, Keravat, New Britain, March 1950 (*G. S. Dun*). British Museum (Nat. Hist.) Collection.

Paratypes. Four females same data as holotype.

This species is closely allied to *P. cheesmanae*, but readily differentiated by the structure and relative length of II and III antennal segments, the more uniform coloration, cuneus with pallid base and apex, membrane much darker and also by the dark spots of the abdomen.

It is dedicated to Mr. G. S. Dun, Government Entomologist to the Territory of Papua and New Guinea.

OBSERVATIONS ON THE LIFE-HISTORY OF THE RED LOCUST, *NOMADACRIS SEPTEMFASCIATA* (SERV.) IN THE SOLITARY PHASE.

By G. F. Burnett.

Late of the *International Red Locust Control Service, Abercorn, Northern Rhodesia.*

The observations recorded in this paper were made between June 1948 and May 1949 in the Milepa centre of the Rukwa outbreak area of the Red Locust, *Nomadacris septemfasciata* (Serv.), in Tanganyika Territory (Lat. 08°04′S., Long. 31°57′E.). Two separate populations of locusts were studied and these are referred to as the "Niombe" and the "Airfield" populations. The exact localities in which they were found, their behaviour, and their phase, are given and discussed in another paper (Burnett, 1951). Both populations were close to phase *solitaria* in 1948 and their offspring may be considered almost extreme *solitaria*. The biometric ratios are given in Table I. The seasonal life-histories of both populations were identical.

TABLE I.

Biometric ratios of the two populations studied : (*a*) Ratio of elytron to femur length, (*b*) ratio of length of elytron in the two sexes.

Population	Size of sample	Male E/F	Female E/F	E female / E male
"Airfield" parent (Oct.–Nov. 1948)	M 16 F 4	1·92	1·91	1·135
"Airfield" offspring (April 1949) ...	M 112 F 78	1·84	1·82	1·210
"Niombe" parent (Oct.–Dec. 1948)	M 103 F 24	1·91	1·88	1·158
"Niombe" offspring (April 1949) ...	M 78 F 65	1·87	1·85	1·216

All measurements made as laid down by the 4th International Locust Conference (Egypt, 1937).

The mean density of locusts varied from a loose swarm in July 1948, to 78 per acre in November and down to 20 at the end of December 1948. The offspring in April 1949 were in a few places as many as 400 per acre but on the average about half this density. Except in the middle of October 1948, the locusts at the Airfield were always less dense than those at the Niombe.

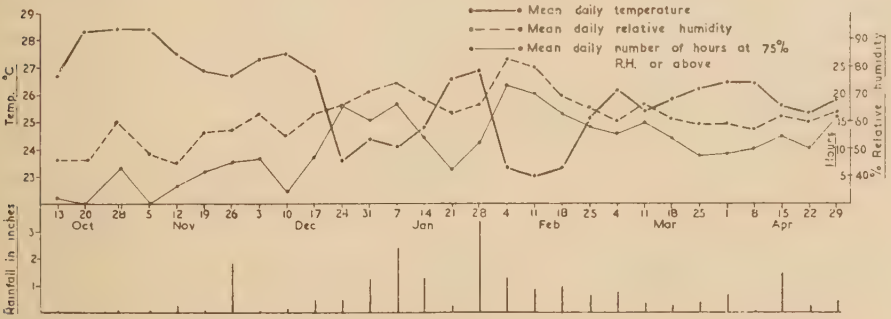

Fig. 1.—Meteorological records, Milepa, 1948/49. The data are plotted opposite the middle date of each seven-day period. The rainfall is shown as weekly totals.

Standard meteorological readings were made at a point less than a mile from the Airfield population and some three miles from the Niombe population and these are summarised graphically in fig. 1. The mean daily number of hours at 75 per cent. R.H. or above have been taken to be a measure of the total exposure to moist conditions and these data are plotted in fig. 1.

Sexual Maturation of Females and Oviposition.

Development of eggs.

Females were dissected at intervals in order to follow the progress of maturation, but specimens were scarce and only one or two at a time could be examined ; maturity was estimated by taking the mean length of ten eggs, five on each side (Table II). The most mature eggs were found on 29th November ; these were 6·5–7·1 mm. long (mean 6·8 mm.), orange, fat and oily in appearance. On no occasion were recognizable eggs found at obviously different stages of development, and it seems that *Nomadacris* lays one batch of eggs before the next shows any visible development. These data show that development of the eggs is probably rapid but occurs

TABLE II.

Development of ovaries and eggs, October–December 1948.

No.	Date	Locality	No. of specimens	Condition of ovaries and eggs	Mean length of egg in mm.
1	25/10/48	Milepa	3	Mass of tubules ; no individual eggs	—
2	11/11/48	Milepa	1	No change	—
3	16/11/48	Milepa	1	Eggs white	2
4	17/11/48	Milepa	1	Eggs white	2
5	19/11/48	Milepa	1	Eggs thick, translucent, a rich orange	6·3
6	22/11/48	Milepa	1	Eggs thin, yellowish ...	5·75
7	22/11/48	Milepa	2	No individual eggs visible ...	—
8	21/11/48	North Rukwa	4	No individual eggs visible ...	—
9	24/11/48	Milepa	2	Eggs thin, yellow	4·3 4·1
10	24/11/48	Milepa	1	Eggs thin and yellowish ...	5·4
11	27/11/48	Milepa	1	Eggs fat, orange, abdomen distended	6·5
12	27/11/48	Milepa	1	Eggs thin and yellowish ...	4·4
13	29/11/48	Milepa	2	(Copulation commenced in afternoon — specimens caught early morning.) Eggs fat, orange. Abdomen distended	6·8
14	6/12/48	Milepa	1	Spent female—1 egg remaining, 6·4 mm. long. Second batch not visibly developed	—

very irregularly throughout a *solitaria* population, *e.g.*, compare 4, 5 and 7 in the Table. The specimens from the North Rukwa (8) are interesting for they came from an aggregation of locusts, biometrically close to *solitaria*, but so dense as to merit the description of " swarms " and which seem to have been all in much the same stage of maturity, with none of the colour changes described below. These locusts however started to copulate about the same time as those at Milepa.

Number of eggs.

Copulation commenced in the field on 29th November, although the first spent female was not found until 6th December ; it continued until populations fell sharply at the end of December, but odd locusts were found as late as mid-February. No eggs were found in the field, owing to the sparseness of insects. Five females confined in a small cage (18×10×15 ins. high) together with seven to five males, laid eleven pods over a period of more than five weeks. One female died when full of eggs and these eggs are included in the following calculations : twelve pods contained, on the average, 76·1 eggs (minimum 47, maximum 133). This gives an average of 182·6 eggs per female. One of these had died, empty, ten days before the others and possibly would have laid another pod. There is likely to be considerable divergence between the fecundity of locusts in cages and in the field, but the period of time during which these caged locusts were laying coincided with that of active breeding in the plains. The number of eggs per pod is fifty per cent. greater than figures given for locusts in swarms (Michelmore & Allan, 1934) but the numbers of both pods and eggs agree well with those for caged locusts given by Faure (1935).

Interval between ovipositions.

Each batch of eggs starts visible development after the previous pod has been deposited. The caged locusts referred to above laid either two or three pods each between 8th December and 15th January, *i.e.*, in 38 days. In the case of those which laid three pods, if one pod was laid soon after 8th December and, as is likely, death did not ensue as soon as the last pod was laid, the other two pods were deposited at intervals of somewhat less than 19 days, probably more nearly two weeks. Table II shows that the first female with visibly developing eggs was taken on 16th November, 13 days before copulation and 20 before the first spent female was recorded. This is in fair agreement with the figure obtained for the intervals between egg pods (19 days or less) and with Faure's (1935) figure of 15 days between layings for ph. *gregaria*.

Duration of Nymphal Instars.

Figures for the length of each instar are in some cases approximate because, when hoppers became scarce in the latter stages, the first individuals to reach each new instar were too few for any to be collected. As a check, specimens of the last three instars were captured in the field and kept until they had undergone at least two ecdyses. In common with previous investigators, a very high mortality was found amongst hoppers in cages. Considerable variation was found in the number of days between moults even with locusts kept in the same cage. The data obtained are summarised below :—

	Egg	Instar						
		1	2	3	4	5	6	7
Duration (days)	39–46 (prob. 42)	7	7–8	10	12	10–11	11–17	14–17

The lowest figure for the duration of the egg stage is calculated from the date when the first spent female was found (6th December) and from the finding of a single second-instar hopper on 21st January, which must have hatched on or before 14th January. The highest figure is the interval between the night after copulation started and the occurrence of sudden and widespread hatching on 17th January. A duration of about 42 days is considered the most probable—from 6th December to 17th January. Any of these periods is considerably longer than has been recorded for locusts in swarms (Smee, 1936 ; Faure, 1935). Soil temperatures were not taken every day, but a number of maximum and minimum temperatures were taken as well as spot readings. Minimum temperatures ranged from 15·3°C. to 25°C. and maxima from 26·0°C. to 39·8°C. (at 2 ins. deep), varying according to situation as well as the weather. The mean daily temperatures all lay between 23·8°C. and 31·6°C. and the mean temperature to which the incubating eggs were exposed is estimated to have been in the range 26–28°C. It is possible that hatching was delayed or that the earlier eggs were destroyed by the drought. If so, the effect was remarkably complete, for the hatching took place simultaneously over a wide area. The drought broke on 19th December and if all eggs laid before this died, the incubation period would be about 29 days.

The figures of ten and 12 days for the third and fourth instars are probably too high. They did not fit in with the otherwise regular increase in the duration of each instar (recorded for *gregaria* also, by Faure, 1935) and moreover the total for all the minima given is 71 days from hatching to adult, whereas the first fliers appeared in the field 67 days after the first general hatching. The difference of four days is accounted for if the real minima for stages 3 and 4 were eight and ten days respectively.

There are no published data on the length of hopper development of *solitaria* Red Locusts. *Transiens* hoppers have been calculated to take about 65 days in a part of the Rukwa and in a season when air temperatures were considerably lower than at Milepa (own observations). Faure (1935) suggests 64 days in a hot, dry summer in South Africa and Smee (1936), for caged hoppers obtained from invading swarms in Nyasaland, gives an extraordinarily long first instar (22–26 days) which suggests that the extra instar was present and unnoticed, although more than three weeks is exceedingly long even for the two stages together. For the last five instars, Smee gives a total of 42 days (minimum) and 56 days (mean). At Milepa the minimum time for the last five instars was 53 days (*i.e.*, 67 minus 14, the duration of the first two instars). That this is fairly close to the mean is indicated by consideration of the rather scanty data obtained in cages. Hamilton (1936), using phase *gregaria* hoppers in the laboratory, obtained mean figures for the total life history of 52 and 52·7 days respectively for males and females, and minima of 45 and 49. These should be compared with 67 days minimum for *solitaria* hoppers in the field at Milepa. Hamilton's figures were obtained at constant 26·7°C. and 75 per cent. R.H. The mean air temperature at Milepa was 24·8°C. (mean max. 30·9°C.) and R.H. 68 per cent. (but probably much higher in the grass where the locusts lived). The mean temperature for Smee's observations was 28·4°C. (32·8°C. max.). Thus conditions at Milepa were cooler than for the other two sets of observations. However, when Hamilton raised the temperature by 5·5°C. it made little difference to the mean nymphal period and it is apparent that the extra instar in the solitary phase increases the time spent in nymphal development. The extended period spent in the egg might also be due to a lower rate of development inherent in the solitary phase.

Morphological Changes during Nymphal Development.

Number of instars.

So far as is known, swarming Red Locusts invariably pass through six hopper instars (Mossop, 1933 ; Michelmore & Allan, 1934 ; Faure, 1935), one more than the

Migratory, Desert or Brown Locusts (Uvarov, 1928). During campaigns, reports have often confused the instars present and so a look-out was kept for suitable characters by which they could be separated. These were found in the numbers of antennal segments and of vertical bars in the eye, the latter being readily counted under a low-power lens. It was soon evident that there was an irregularity in the development of these locusts and that an extra instar was present. The gradual development of the eyes, wing rudiments and genitalia is shown in figs. 2–5 ; and the numbers and variability of the eye stripes and antennal segments are given in Table III. All these features differ from those given for *gregaria* hoppers by Michelmore and Allan (1934). Instars one and two resemble more or less closely the corresponding instars in the other phase ; the sixth and seventh instars of *solitaria* correspond fairly closely to fifth and sixth of *gregaria*, the wing rudiments being turned up over the back, so the extra instar is interpolated prior to this. It may be noted that Bredo (1938) correctly described the first five instars of the solitary phase but missed one of the last two, probably the sixth (penultimate). His counts for the antennal segments of all but the first three instars do not correspond with those obtained in the present work.

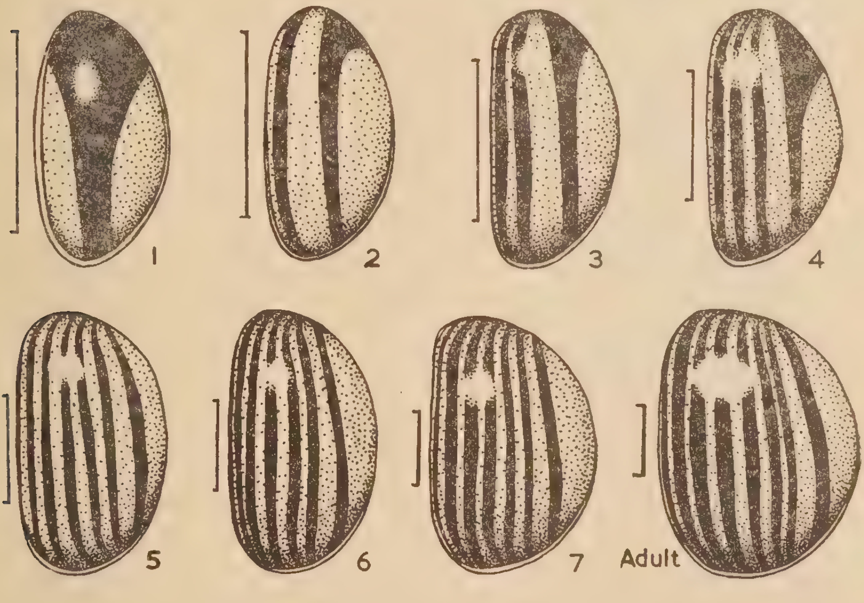

Fig. 2.—The development of the left eye in successive nymphal instars and the adult of ph. *solitaria*. The lines represent 1 mm. in length and give the scale for the female. The fifth instar shows a " rim " stripe—an extra short and narrow bar along the anterior edge.

Eye stripes (fig. 2).

The dark vertical stripes of the compound eye are connected above by a single horizontal bar in the shape of a segment of circle and therefore cannot be shown in fig. 2 which shows the number of vertical stripes present in each instar. The last stripe may be almost obsolete, especially ventrally, and in the early instars may be broadened above (fig. 2, instars 2–4). The " rim " along the anterior margin in fig. 2, instars 5 and 6, no doubt represented the extra stripe which is inserted at the next ecdysis ; it was always narrow and is not counted in Table III. In the adult there was never a rim stripe quite like this, all stripes being full length, even if some were narrow. The shape of the eyes is correlated with the number of stripes. Fig. 2,

TABLE III.

Characters of the different instars of the ph. *solitaria*, compared with those of ph. *gregaria* hoppers (Michelmore & Allan, 1934).

solitaria, Milepa, 1949							*gregaria*, N. Rhodesia, 1932 (M. & A., 1934)	
		Eye stripes			Antennal segs.			
Instar	Number examined	Number	Per cent. of sample	Per cent. with rim	Number	Per cent. of sample	Number of antennal segs.	Instar
1	48 including both sexes	Solid or very faint single bar	100	Nil	13 14	98 2	13 (14, 15)	1
2	32 male 37 female 1 indet.	Solid 2	24 76	Nil	16 17 18	1·5 97 1·5	17 (18, 19)	2
3	34 male 31 female	Obscure 3	5 95	4·5	19 20	44 56		
4	45 male 32 female 2 indet.	Obscure 4 5	4 95 1	8	20 21 22 23	1·5 81 16 1·5	20	3
5	27 male 43 female 4 indet.	Obscure 5 6	4 85 11	21	22 23 24 25 26	1·5 72 15 10 1·5	22	4
6	30 male 27 female 2 indet.	5 6 7	3·5 84 12·5	42	24 25 26 27	3·5 64·5 22 10	24 (25)	5
7	25 male 29 female 4 indet.	6 7 8	5 84 11	36	26 27 28 29	4 46 35 15	26	6
Adult	50 male	7 8 9	26 72 2	Nil	27 28 29 30 31	2 26 64 6 2	27	Adult male
	50 female	7 8 9	6 92 2	Nil	28 29 30	8 76 16	27	female

instar 5, is abnormal in that it has six stripes and is the same shape as instar 6; normal specimens with five stripes were intermediate in breadth between fourth and sixth instars. Those locusts which died in ecdysis appeared to have the normal number for the instar just passed through but when the old cuticle was prised off, the extra stripe was found underneath, at the anterior border. The single stripe of the first instar was visible in a minority only of the specimens and to see it the light had to be at a favourable angle. A small percentage of later instars had eyes so heavily pigmented that the stripes could not be counted with certainty ; this is the normal condition in *gregaria* and in *transiens* nymphs.

It will be seen that the number of eye stripes is not perfectly correlated with the number of instars passed through, in contrast with the report of Mukerji & Batra (1938) on *Schistocerca gregaria* (Forsk.), as has been noted already by Roonwal (1940, 1946). There is, however, sufficient connection between the number of stripes and number of instars and between instars and phase for us to expect the number of stripes to indicate the phase, as has been pointed out by Volkonsky (1938). Since the shape of the eye varies with the number of stripes, the ratio of length to breadth of that organ might be of use as a phase character in *Nomadacris*, as it is in *Locusta* (Thomas, 1941).

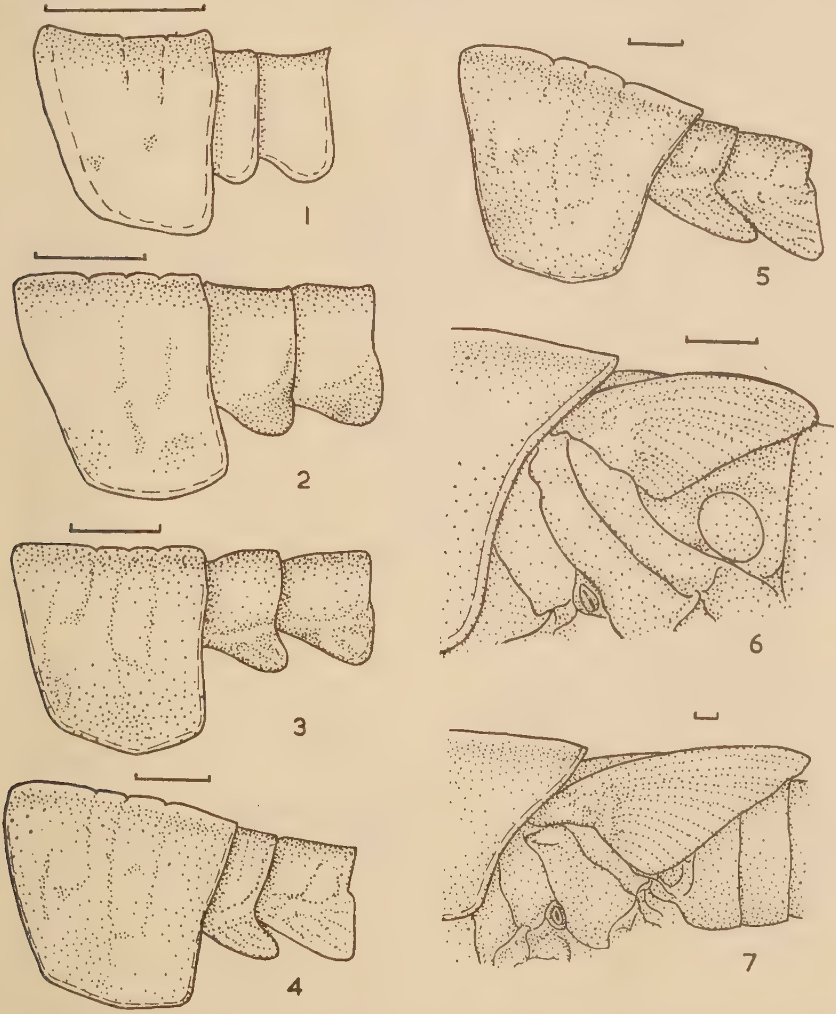

Fig. 3.—Development of wing rudiments and pronotum in successive instars of ph. *solitaria*. The lines represent 1 mm. in each case and give the scale for female specimens. Hairs are omitted.

Number of antennal segments (Table III).

The first and second instars of *solitaria* have the same number of segments as in those of *gregaria*. Subsequent instars do not correspond exactly with any *gregaria* instars but have more segments than the nearest equivalent instar as determined by general development. The number is also much more variable, some divisions being more distinct than others, or even not complete ventrally, in which case the division was not counted. Variation was particularly great in the third instar. In all instars segment six was the most likely to be incompletely divided and obviously the seat of most active division, followed by 5, 7, 8 and 11, in that order of frequency. Occasional sutures were crooked, usually accompanied by other signs of regeneration such as a subnormal number of segments. Only one male adult out of the 50 examined, and not one of the 50 females, showed the total number of segments normal for *gregaria* (27). All the rest had more than this, 29 being the commonest number. The locusts with fewer than 29 segments had only seven eye stripes.

Pronotum and wing buds (fig. 3).

The main features of each instar may be summarised as follows :—

First.—Wing rudiments present merely as the rounded lower margins of the meso- and metathorax. Hind margin of pronotum emarginate.

Second.—Demarcation of wing-pads from tergites wanting or very indistinct: mesothoracic lobe produced downwards but not at all backwards, metathoracic lobe rounded. Pronotum emarginate behind.

Third.—Mesothoracic lobe produced slightly backwards, rounded. Metathoracic lobe usually with rounded ventral and posterior edges, demarcated from tergite by a ridge ; traces of longitudinal striation present. Pronotum not lobed medially behind, very slightly emarginate.

Fourth.—Forewing produced backwards but not to middle of metathorax. Rear margin of metathoracic wing lobe straight and with a definite notch where its anal angle meets metathoracic tergite ; anal area of this wing just discernible as a distinct area. Pronotum behind slightly lobed medially, not emarginate.

Fifth.—Wing rudiments more distinctly demarcated. Forewing produced down and rearwards to reach the middle of metathorax. Hind wing with straight edges and anal area very distinctly separated by a ridge from rest of wing. Pronotum strongly lobed posteriorly and distinctly arched dorsally when seen in profile.

Sixth.—Wing buds reflected over back as in fifth stage *gregaria*. Forewings reach hind edge of first abdominal segment. Pronotum less arched than in previous stage, prolonged backwards.

Seventh.—Forewing covers abdominal segments 1–3 and part of 4. Pronotum strongly prolonged posteriorly, not arched in profile.

When compared with the figures and description of Michelmore and Allan (1934) it will be seen that the pronotum in *solitaria* is never produced backwards to the same extent as in *gregaria* and in several instars it is strongly arched. Instars two to seven correspond quite closely to the six *gregaria* instars, the first *solitaria* instar having less developed wing rudiments than any *gregaria* hopper.

Male genitalia (fig. 4).

Michelmore and Allan (1934) give purely diagrammatic figures of abdominal terminalia in ph. *gregaria*. Those of the male cannot be certainly correlated, instar for instar, between the phases. The first two instars in *solitaria* are similar, except for size and the relatively prominent cerci in the younger, and the instars two to seven correspond only approximately to the normal six instars in *gregaria*. In

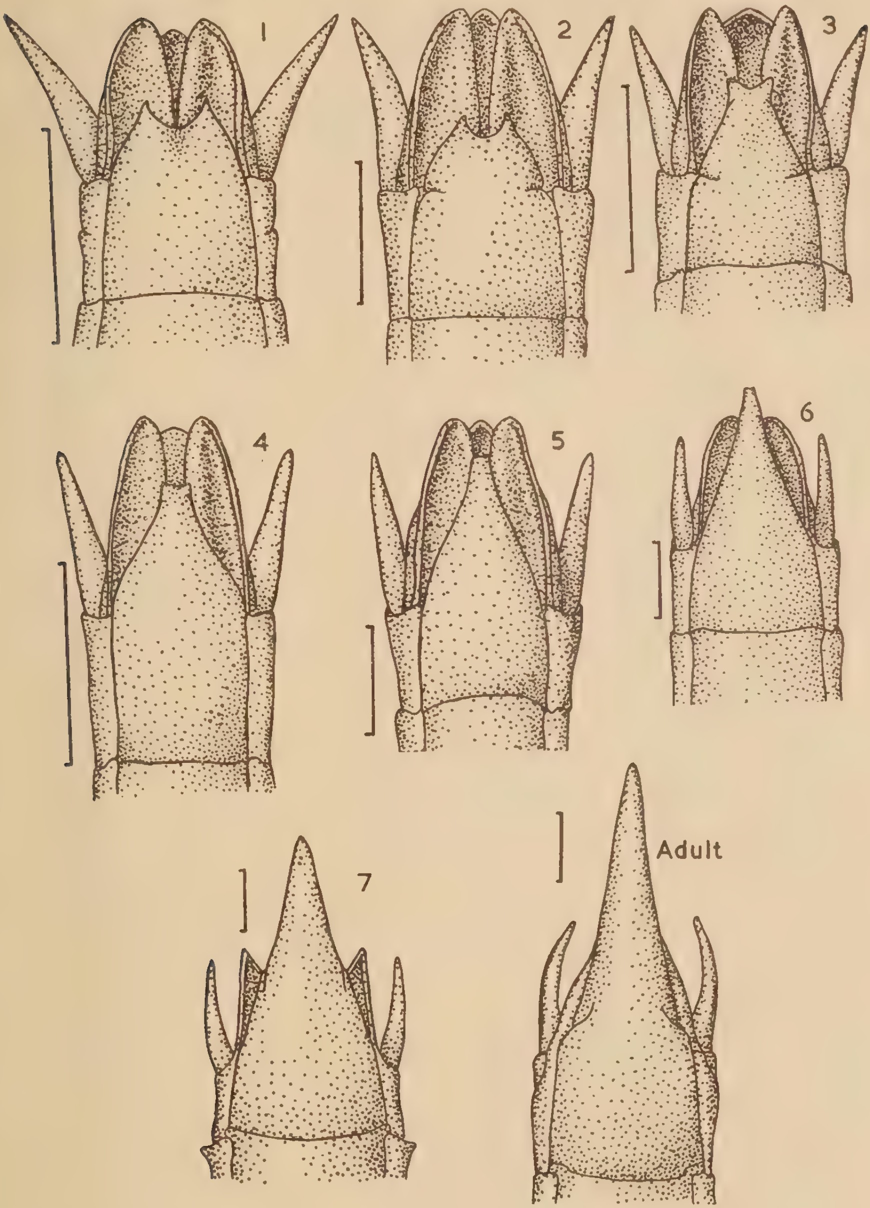

Fig. 4.—Development of the male genitalia in successive instars of ph. *solitaria.* The lines represent 1 mm. except in the first two instars where it is equivalent to 0·5 mm. Hairs are omitted.

solitaria the ventral plate is relatively longer compared to the paraprocts and is less deeply forked. In the last nymphal instar the male genitalia are almost the same as in the adult.

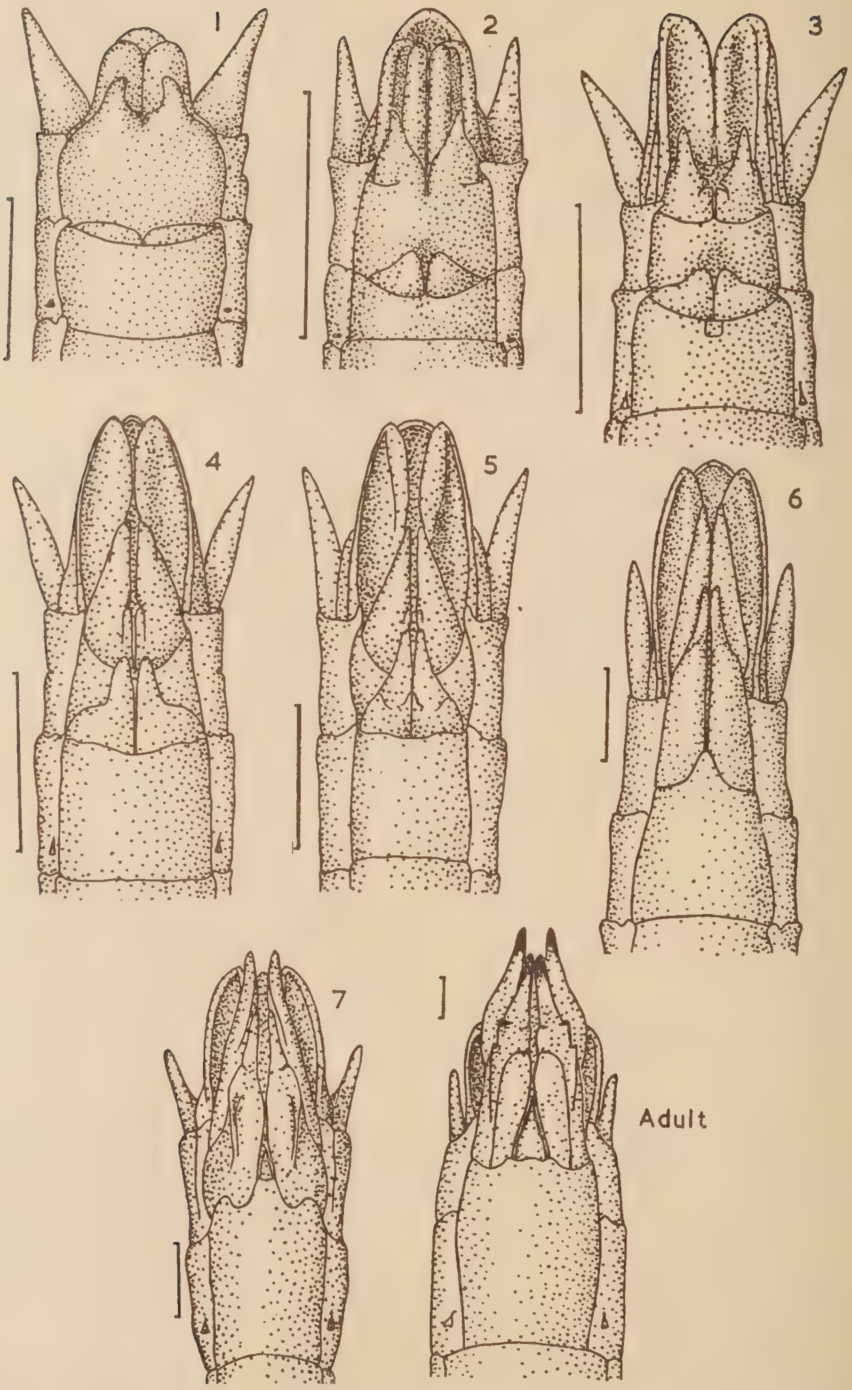

Fig. 5.—Development of female genitalia in successive instars of ph. *solitaria*. The lines represent 1 mm. except in the first stage where it is equivalent to 0·5 mm. Hairs are omitted.

Female genitalia (fig. 5).

In contrast with the male these develop more rapidly in the *solitaria* than in *gregaria*. Even in the first instar, the ventral valves are visible as slight projections on the posterior edge of the eighth abdominal sternite, whereas in the *gregaria* they do not appear until the second instar. In the third instar *solitaria* hoppers, the inner valves have commenced to bud off from the inner edges of the posterior coxosternites but not until the next instar do they achieve the condition shown in third instar *gregaria*. The same applies to the two pairs of principal valves, the third instar *solitaria* being in respect of the female genitalia truly intermediate between the second and third stages of *gregaria*. The last four instars are quite closely equivalent to the last four *gregaria* instars. There are quite considerable changes in the last moult.

Size.

It has not been possible to make an adequate series of measurements but it was found quite feasible to allocate insects to the correct instar by size alone, with only one error in some four hundred individuals. Comparison with an incomplete sample of *transiens* hoppers showed that there was no specimen present in the latter which corresponded to the third instar *solitaria* but that the second instar *solitaria* was noticeably smaller than the corresponding *transiens* hoppers (there were no first instar present in the sample), while third instar *transiens* was close in size and characters to fourth instar *solitaria* and very close to the *gregaria* third instar as figured by Michelmore & Allan (1934). Sexual dimorphism in size was not obvious until the sixth instar but would doubtless be revealed in earlier instars by biometric analysis.

Intermediates.

Individuals combining characters of two instars were most common in the later instars (Table III) and in nearly all cases, except the third instar, variation was towards a later instar, and in several characters simultaneously. Intermediate males with extra eye stripes might be as large as normal females but there was no difficulty in allotting them to their correct category on sight. Even large males were smaller than normal males of the next instar and in the case of the last two instars the degree of development of the wing-pads was quite unmistakable. There was less variation in abdominal terminalia and wing buds than in eye stripes and antennal segments. Third instar hoppers were more variable than the others and varied in both directions, *e.g.*, when the wing rudiments were undoubtedly of third instar, the genitalia might resemble those of the second.

Discussion.

From the remarks on the relative sizes of the early instars of *transiens* and *solitaria* it will be evident that the extra instar is not merely a duplicate of another. Except for size, the second instar *solitaria* closely resembles the same instar in *transiens* but the third is intermediate in the character of the female genitalia, number of antennal segments and size between the second and the third *gregaria* (or *transiens*). It is also the most variable instar and it varies in both directions. The fourth *solitaria* instar is not unlike the third of *transiens* so far as wing buds and genitalia of both sexes are concerned. Although development of male genitalia and wing buds might point to an extra instar before the first, it is probably safe to say that the extra instar is effectively the third (*solitaria*) but that its occurrence has so upset the normal course of development that the whole life-history has been affected and the later instars in the two types of development cannot be accurately equated. This, although not unexpected, differs from the findings of Key (1936) in *Locusta migratoria migratorioides*, of Zolotarevsky (1933) in *L. m. capito* and from most of the other examples collected by Roonwal (1946) in his summary, the extra instar in those cases always being a duplicate of an earlier one. In certain recorded cases where there were two extra instars in the life-history these were both duplicates, *e.g.*, an extra third and an extra fourth in addition to the normal third and fourth in certain examples of *S. gregaria* (Mukerji & Batra 1938).

Roonwal (1940, 1946) comes to the conclusion that extra instars in Acridids follow a definite rule : in the case of a normal six instar life-history the extra instar is usually a duplicate of the fourth (one before the wing rudiments become rotated upwards). It will be obvious that the Red Locust is an exception to this rule ; the extra instar is not an accurate duplicate of any other and in any case occurs too early in the life-history, being on the whole intermediate between the second and third instars of *gregaria*. It is certainly not a duplicate of the normal fourth.

The occurrence of extra instars has been reported only in a very small proportion of the population of other species except for the subspecies *capito* of *L. migratoria* in Madagascar in which all the females and 75 per cent. of the males underwent the extra moult (Zolotarevsky, 1933). It is believed that in *Nomadacris* all, or almost all, *solitaria* individuals had the extra instar.

Mortality in cages was too heavy to follow the life-history of a single specimen right through, but of some seventy individuals kept together in a small cage, the majority, if not all, passed through seven instars, although by the second instar they were very dark and as heavily marked as hoppers from bands of *transiens*. The adults from the field were of very uniform dimensions (Burnett, 1951), the frequency distribution curve for the E/F ratio being almost exactly symmetrical. If any considerable proportion of locusts had the shorter life-history but were as adults of the same size as the others, we should expect a marked bimodality of dimensions in the middle instars due to the accomplishment of the same amount of growth in six steps instead of the seven. It has not been possible to make sufficient measurements for an adequate test, but two reasons may be given for doubting that a considerable proportion of the Milepa locusts passed through the six-instar life-history. All second instar hoppers examined were obviously smaller than those from a sample of *transiens* hoppers, and this indicates that the difference in size between individuals undergoing the two types of life-history would be large but as mentioned on p. 483 there was no difficulty in sorting out all hoppers into their correct instars by eye alone, which would not be likely if there was any confusion due to the presence of locusts passing through both types of life-history.

The unusually large " intermediates " of the later instars do not fit a six instar history, for it is in the earlier instars that large hoppers would represent locusts due to metamorphose in six moults and there is no overlap in numerical characters in these early hoppers (Table III) as would be probable if there were true intermediates. Further, only one adult in a hundred had the 27 antennal segments characteristic of *gregaria* locusts. The weight of the evidence therefore is that, unlike previous examples in the literature, *Nomadacris* passes through seven larval instars in the *solitaria* phase and seems to be unique in having a constant difference in the number of instars in the two phases.

Significance of the extra instar.

The occurrence of seven instead of six nymphal instars may suggest a generally lower metabolism of *solitaria*. It is also reflected in the increased size of locusts of this phase—it may be remarked that extra instars are frequently more common in the females of various species of Acridids (Zolotarevsky, 1933 ; Volkonsky, 1938 ; some examples quoted by Roonwal, 1946), which are usually larger than males. The extra instar in *Nomadacris* is remarkable in appearing so early in the life-cycle and is presumably determined by the phase of the parent. Its occurrence should prove a good test of the presence of true *solitaria* locusts in the field ; also the change from one type of life-history to the other may suggest the direction of phase change in a population several weeks before biometrical measurements can be made on the young adults.

The increased period spent in development may have an influence on the process of phase change. It permits a more prolonged action of the influences affecting phase change in either direction and the extra moult may increase the chance of such changes expressing themselves in the body dimensions of the adult.

Colour Changes.

Nymphal coloration.

The types of coloration shown by hoppers of the Red Locust in the *transiens* and *solitaria* phases have been described several times (Faure, 1932, 1935 ; Michelmore & Allan, 1934 ; Lea & Webb, 1939). The coloration observed at Milepa was extremely variable and since it could not be correlated at all closely with phase it will not be described in detail. The principal colour varieties are described and excellent coloured plates given by Faure (1932). Some of the specimens captured at Milepa had even less dark pigment than those figured by Faure, the only black marking being a broken stripe beneath the eye (genal stripe of Michelmore & Allan, 1934).

All first instar hoppers were similar and differed from the *gregaria* type only in being paler and less heavily marked, the general appearance being grey instead of black. From the second stage onwards the green, extreme *solitaria* type of Faure (pl. XVI, fig. 27) and a similar type with a pure yellow ground colour, were abundant. In addition an orange type was common. This resembled pl. XVI, fig. 30 of Faure but was much brighter, especially in the early instars. It was as brightly orange as swarm-type hoppers but the black markings were much reduced and the bright chrome-yellow of the pronotum replaced a duller yellow. All three colour varieties were found together and all showed great variation in the extent and intensity of markings.

As the season progressed, the percentage of orange hoppers fell from over 60 in second and third instar to under 20 in the sixth. The proportion of all varieties with reduced markings increased at the same time. Lea & Webb (1939) in 1937 noted a similar decrease of the orange type. The characteristic lateral abdominal band of the *gregaria* phase (Michelmore & Allan, 1934) was frequently well marked although broken-up, and was carried over into the young adult. It was noted in caged specimens that the amount and intensity of markings on the abdomen of fledglings was directly related to the degree of pigmentation present in the last instar hopper. A heavily pigmented abdomen was usually, but not invariably, associated with dark and distinct tegminal bars. Nymphs captured in the field and confined together in a cage, although not crowded, accumulated pigment in their later instars, sometimes in the form of prominent markings (orange and yellow colour varieties) or as a general duskiness distributed without affecting the markings (particularly in the green specimens). This pigment became obvious in the adult, and such green hoppers gave adults as strongly marked as those from yellow or orange nymphs which had prominent abdominal spotting. All were biometrically *solitaria*. The adult coloration was visible beneath the last nymphal cuticle 24 or 28 hours before ecdysis, and newly emerged adults were completely marked except for the tegmina, which were uniformly pale. The pattern was still very faint over an hour after eclosion, but almost complete half an hour later, continuing to deepen for a couple of days. The pink of the hind tibiae took several days to develop. The final moult almost always took place between 10.00 and 12.00 hours.

Adult coloration.

Published descriptions of the colour of *solitaria* adults and of its changes with the season are less complete than for the nymphs (Faure, 1932, 1935 ; Lea & Webb, 1939). The last authors give a brief and general record of the changes during the year but a more detailed account is given here. Colour changes in swarms are described by Mossop (1933) and Faure (1935).

Colour of young adults, dry season.

What may be termed the full basic colour pattern (except for the red of the hind wings) was developed within two days of the final moult. It is therefore described here in some detail, from specimens obtained in 1949.

General appearance.—Pale yellow-buff with obscure markings.

Head.—Buff. Stripes of vertex yellow-buff. Post-ocular stripe pale- or red-brown, or olive, continuous between the eyes with the olive-green borders of median pair of frontal ridges. Borders of lateral pair browner and confined to frons. Clypeus and labrum brownish buff, finely and shallowly punctate. Former has basal orange to biscuit coloured suffusion on frons between the ridges. Genal wedge olive-green, half width of eye at base, border buff or creamy. Mandibles with greenish-brown facets, pale ridges. Other mouth parts pale buff. Eyes with eight, rich brown stripes on yellow-buff ground. Ocelli unpigmented. Antennae pale buff proximally, changing to yellowish distally.

Pronotum.—Dorsal stripe straw-coloured. Lateral fascia, varying shades of purplish brown. Sometimes with reddish punctations between posterior one third of lateral bands, otherwise buff.

Thoracic pleurae.—Buff. Very faint biscuit-coloured suffusion deepening to greyish or greenish in dark specimens. Buff ventrally.

Abdomen.—Pale buff, brown-tinged. Hopper markings persist as an indefinite purplish dorsal stripe, whitish lateral stripe and spots on the tergites. These may be almost absent or very heavy and distinct.

Legs.—First and second. Greyish above, buff beneath. Tibiae faintly purple on outer aspect. Tarsi rather brown, especially arolia. Tarsal pads orange-brown. Claws dark brown. Third. Buff. Keels of femur (except ventral) brown-lined. Herring-bone pattern not marked. Outer face of tip of femur greyish-white with brown crescent. Tibia brownish-buff, purple-pink between spines, which are white with black tips. Tarsi purple above, pads brown, tinged grey. Claws black-tipped.

Tegmina.—Buff. Bars usually indistinct in outline, sometimes almost obsolete, those near the tip being represented only by pigment on the veins ; the veins between the bars whitish. Main subcostal and median veins reddish-brown. Subcostal and anal areas very pale, yellowish in parts.

Hind wings.—Clear and colourless, veins very pale brown.

Traces of the small brown spots which are found on all hoppers persist on fledglings, principally on the legs, and tend to be purple-tinged.

Dark specimens differed from the pale ones principally in the deeper colour and more definite markings of the tegmina and in the increased density and the tendency to olive-green of the brown markings on the head. All specimens from the field were considerably paler and less reddish than Faure's pl. III, fig. 18. Caged insects developed a rosy flush on the head, body, hind femur and anal area of tegmina (*cf.* Michelmore & Allan, 1934). This never appeared in the field and was probably due to the relatively crowded conditions. It may correspond to the red body colour of ph. *gregaria.*

Within a month of emergence a very faint hint of pink appeared on the hind wings. By the second half of May (about seven weeks after the first adults appeared and when the majority were four to six weeks old) all specimens in the field had an appreciable amount of purple-red on the hind wings. The orange suffusion on the face, the biscuit colour of the thorax and the nymphal markings of the abdomen had all faded almost completely. The appearance of these locusts was now, except for the smaller amount of red on the wing, identical with that of their parents the previous June. The description of changes that took place from June to January is based on these locusts, the generation of 1948.

At the end of June the full dry season pattern was developed. The general appearance had changed very little, the markings of the thorax and head were somewhat duller and the pinkish-purple of the hind wings fully developed and extended over the basal quarter of the wing. Both abdominal markings, except for an incomplete dorsal stripe, were completely obsolete.

There was no further change until the beginning of November when breeding coloration started to develop and changes continued until death.

Maturation changes.—The most conspicuous changes involved firstly, rapid darkening and increase in definition of the bars of the tegmina ; these became almost black in those specimens in which changes were most complete, the veins between the bars appearing white by contrast, the anal and sub-costal areas became yellower and later brownish. Secondly, the purple pink of the hind wing extended to include up to 50 per cent. of its area (more usually 30–40 per cent.) and changed to an obvious crimson and even spread on to the dorsum of the meso- and metathorax and the first two abdominal segments. Thirdly, the dorsal aspect of the hind tibia and tarsus turned purple-blue ; and, fourthly, a pink suffusion reminiscent of that in the fledgling, but more widespread, appeared in the following areas : three broad vertical stripes between the frontal ridges, the outer pair continuing on to the labrum; two patches on each side of the prothorax between the lateral fasciae, a similar patch on each of the pleurae of the meso- and metathorax ; lines on the outer keels of the hind femur—a fine line on the lower keel and a stripe 1–2 mm. wide on the upper. This colour later turned bright orange and later still, except on the face, was rapidly transformed to, or overlaid by, other pigments—a bright golden-yellow in the male and a warm reddish-brown in the female. The yellow in the male also spread on to the sides of the abdomen, while the reddening of the female was particularly noticeable on the thoracic pleurae. Less conspicuous changes took place in the ground colour and the basic markings. The ground colour tended to become darker—the cream parts becoming buff and the buff and yellow parts turning various shades of warm brown or even orange-brown and gradually deepening. Only the ventral surface of the abdomen and the border of the genal wedge were relatively unaffected. The dark markings of the body tended to become darker although not so much as those of the tegmina, and the outer surface of the hind femora developed a herring-bone pattern. The genal wedge and postocular stripe deepened in colour and became broader, apparently due to the laying down of brown pigment at their edges.

These changes did not take place at a uniform pace throughout the population and many of the males never developed the full golden-yellow. The darkening especially developed more rapidly and evenly among the females. Changes commenced in early November and by the middle of the month some 60–70 per cent. of all locusts could be called " dark " : the bars of the tegmina had firm edges, the deepening of the colour of the wing, of the prothoracic fasciae and of the ground colour of the body had started and the pink suffusion had reached various stages of development but showed no trace of turning orange. The hind tibiae were already purple-blue above. A few males were so dark and sombre in colour as to be strongly reminiscent of dried specimens. The remaining locusts were still pale and with no pink suffusion. Ovary development was very uneven (Table III). When copulation commenced on 29th November nearly all females and some 70 per cent. of males were " dark." The former were more uniform, with the tegmina strongly barred with dark greyish-brown, and the pinkish suffusion well developed. Some males were almost as dark, others very pale and diffusely marked but all readily copulated, a pallid, yellowish male and a dark female, *in copula,* contrasting strongly. No specimens of those examined at the end of November showed any change of the pink suffusion to orange, but locusts were much more conspicuous against their background. Two weeks later changes had proceeded further and some females were almost black. The crimson of the hind-wings was fully developed. The pinkish suffusion had changed to orange and the ground-colour of the legs and body was distinctly brown, especially that of the pronotum, where the lateral fasciae tended to be confluent anteriorly.

After another week or less the breeding season coloration in many specimens might be considered to be at its height, the golden-yellow and the reddish-brown of males and females respectively having suddenly developed. The markings of the body were strongly developed and not yet obscured by the darkening ground colour. A sample of 33 taken on 23rd December were all dark. Later changes may be considered degenerative although locusts continued to feed and mate vigorously. The yellow of the males faded to a dull orange, the corresponding red-brown of the females becoming obliterated ; the markings became dull owing to the deepening and darkening of the ground colour to dull shades of amber and burnt sienna. The labrum often changed to grey and irregular greyish blotches appeared here and there on the frons. The dorsal streak turned brown and darkened but the pale area outlining the genal wedge, which remained a dull buff, was the palest part of the body apart from the ventral surface of the abdomen. The abdomen became an unhealthy looking yellow, plainly visible in flight.

Discussion.

Adults of ph. *solitaria* and ph. *gregaria* of *Nomadacris septemfasciata* differ very little in morphology but it has been believed that the solitary phase is distinguishable by its general paleness (Faure, 1935 ; Johnston & Buxton, 1949). That this is largely a seasonal matter was indicated by Lea & Webb (1939). The real distinction between the phases would seem to be the absence in *solitaria* of the general reddening of the body which takes place in flying swarms within 2–3 months of the last moult (Mossop, 1933) and which tends to obliterate the pale dorsal stripe. This reddening was found in locusts with an E/F ratio of only 2·05 (Gunn & others, 1948). Several authors agree that the colour of the hind wing is more purple in the solitary locust and develops earlier in life (Lea, 1938 ; Lea & Webb, 1939 ; Callaway in Gunn & others, 1948). It has been observed by me that this purple changes to a bright crimson with sexual maturity. The bright yellow of some males which appeared when breeding was well under way resembled the colours developed by breeding swarms (Faure 1935, fig. 19 ; Michelmore & Allan, 1934). There was some individual variation in pigmentation of the pale *solitaria* but this was a reflection of the pigment present in the last hopper instar, and not of phase difference. The maturing adults of the parent generation were arbitrarily classified into " dark " and " pale " forms and the frequency distribution curves of E/F ratio were plotted for each, and no significant difference was found. By the middle of December all locusts were dark.

Colour changes which have been described both in swarms (Michelmore & Allan, 1934) and in *solitaria* (Lea & Webb, 1939) were said to start at the beginning of the rains. In the present case, both maturation of eggs and darkening of the tegmina, etc., were far gone by the time the first significant rain fell on 27–28th November. Very probably there had been no actual precipitation in the areas inhabited by the locusts—certainly the ground had never been visibly moistened. Reference to fig. 1 will show, however, that although there was no considerable rise in mean relative humidity in November, there was a marked increase in the number of hours with high humidity. The amount of cloud also increased. While food was by no means lush, several species of grass produced quite an amount of green shoots and the appetite of the insects increased. Maturity of the eggs and accompanying colour changes may well have been due to the increased ingestion of moisture with food and to exposure to increasing daily periods of higher atmospheric humidity, but they were not due to the actual start of the rains.

Summary.

The populations of *Nomadacris septemfasciata* described in this paper were all of *solitaria* or near-*solitaria* phase.

Sexual maturation as measured by the developing ovaries took place rapidly though irregularly through the population. Its onset coincided with an increase in the daily number of hours of relative humidity at or above 75 per cent., and abundance of green food. Copulation, which started after the first wide-spread rain, took place before the ovaries of many females were completely mature.

Caged locusts gave a mean fecundity of 183 eggs contained in 2·4 pods which is probably a minimum figure. Pods were laid at intervals of about two weeks and each was laid before the next batch had visibly developed.

The period spent in the egg was at least 39 days, at most 46 days and probably 42 days. Adults appeared 67 days after the first hatchings. Both these periods are greater than those recorded in phase *transiens* or *gregaria*.

Solitaria locusts were found to pass through seven instead of six nymphal instars as do *gregaria*. This difference seemed to be constant. The extra instar was probably interpolated between the second and third of *gregaria* but was not an exact duplicate of any other instar.

The number of vertical dark eye-stripes is a convenient and fairly constant indicator of the instar. The number of antennal segments in the later instars and in the adult, and also the number of adult eye-stripes, is greater in the *solitaria* phase.

Amongst the hoppers three main types of coloration were encountered and are briefly described but they could not be closely related to phase. The amount of dark pigment present in late stage nymphs was reflected in the young adult, the appearance of which is described in some detail. Subsequent changes during the dry season are briefly noted, in particular the pink of the hind wing, which becomes faintly visible within a month of the last moult.

The changes in pigmentation which take place during sexual maturation are described in detail. In addition to pronounced darkening of the tegmina which completely changes the appearance of the *solitaria* locust, certain bright pigments are laid down which are later obscured by a general blackening. It is unsafe to classify *Nomadacris septemfasciata* as to phase on colour alone unless the date of capture and its relation to the breeding season in the locality is taken into consideration. *Solitaria* do not normally develop the general reddening of the body which is shown by migrating swarms, even when these are only *transiens* in phase.

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A REVISION OF *DIPAROPSIS* HMPS. (AGROTIDAE, LEPIDOPTERA)

By A. N. Clements.

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The genus *Diparopsis* Hmps. is confined to Africa. During the present century, an insect always identified hitherto as *Diparopsis castanea* Hmps. has become a serious pest as a bollworm of cotton in many parts of that continent. Until recently, the genus was thought to consist of only two species, *D. castanea* Hmps. and *D. tephragramma* B.-Bkr., but differences in the structure of the frontal process of the adult suggested that the former comprised two or more distinct sub-species or species. The present study was undertaken to determine the status of these different forms. It is based on the material in the collections of the British Museum (Natural History) and on additional material obtained through the courtesy of the Government Entomologist, Anglo-Egyptian Sudan, and the Commonwealth Institute of Entomology.

Before pupating, the larva of *Diparopsis* burrows a short distance into the soil and constructs a strong earthen cell lined with a tough silk membrane. The frons of the adult bears a prominent process (figs. 1–6), probably a " cocoon-cutter ", to aid its escape from the cell. In the specimens of *Diparopsis* examined, four distinct forms of frontal process were found, differing in length, degree of flattening, etc. Examination of the male genitalia also revealed four distinct forms, correlated with the differences in frons structure. On the strength of these differences, and further differences in wing pattern, distribution and food-plant, four species were recognised —*D. castanea* Hmps., *D. tephragramma* B.-Bkr., *D. perditor* sp. n. and *D. gossypioides* sp. n.

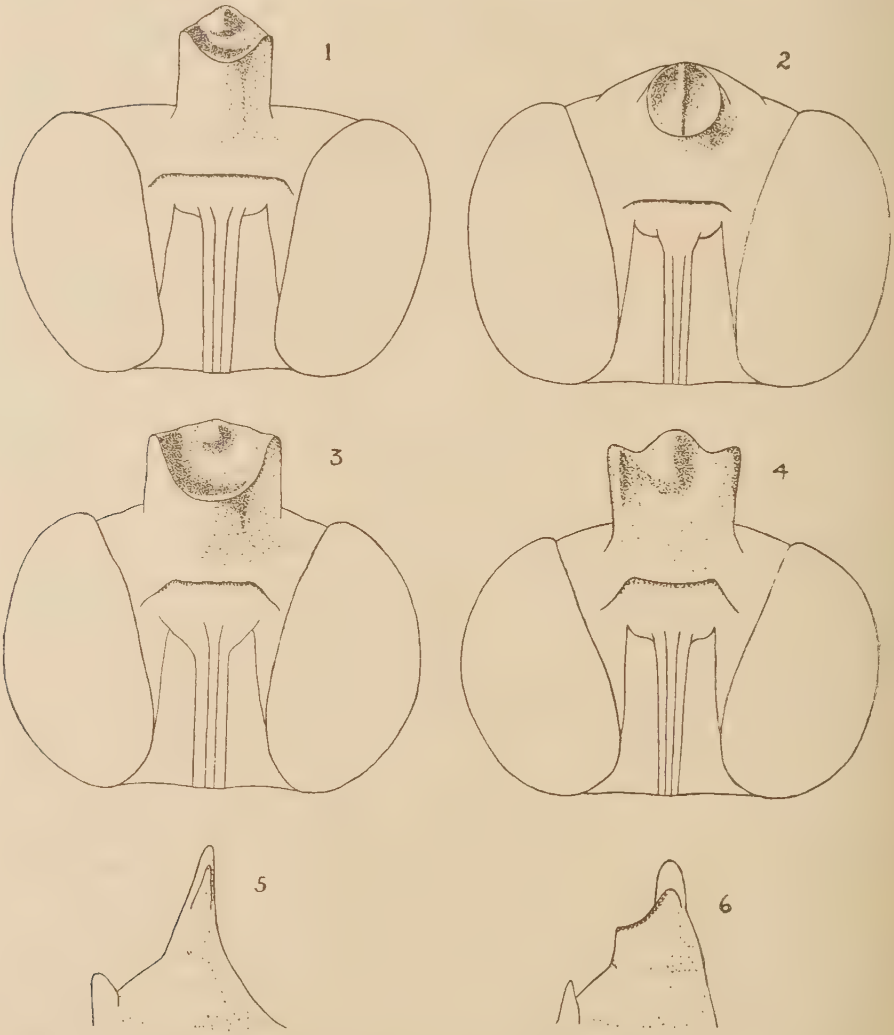
D. perditor sp. n. has previously been confused with *D. castanea* Hmps. The two species have distinct geographical distributions. *D. perditor* is confined to a northern belt extending across the savannah regions from French West Africa to Somalia. With one possible exception, the specimens of *D. castanea* in the British Museum are from a southern belt that includes Nyasaland, Northern Rhodesia, Mozambique and Natal, and it is clear from the literature that this species also occurs in Southern Rhodesia and in various parts of South Africa in addition to Natal. The exception is a specimen labelled " Uganda, H. H. King, 1907 " ; this specimen is presumably the one recorded from Uganda by King (1917), a record which has been widely quoted in subsequent literature. *D. castanea* has, however, never been known as a cotton insect in Uganda, and there are no further records of its occurrence there. Clearly, *D. perditor* and *D. castanea* occupy two belts separated by over one thousand miles. The distribution of *Diparopsis* in Tanganyika is not well known at present. It appears to be persisting on both upland and kidney cotton in Songea (Harris, 1942) and has been reported on *Gossypioides kirkii* (Mast.) up the coast to within fifty miles of Dar-es-Salaam (Jepson, unpublished, 1947). The British Museum specimen of *D. gossypioides* sp. n. was reared from a larva that was found feeding on *Gossypioides kirkii* just north of Lindi. It remains to be shown whether *D. castanea* also occurs in Tanganyika or whether all previous records have been of *D. gossypioides*. *Diparopsis tephragramma* B.-Bkr. appears to be confined to Angola ; its food-plant is unknown.

The two species hitherto confused as *D. castanea* have been called Sudan Bollworm, Red Bollworm and African Bollworm. If English names are to be retained, Sudan Bollworm would most suitably apply to the northern species, *D. perditor* sp. n., while Red Bollworm could be restricted to *D. castanea* Hmps.

The descriptions of *D. castanea* and *D. tephragramma* that follow are modifications of the original descriptions by Hampson and Bethune-Baker, respectively.

Genus *Diparopsis* Hampson.

Proboscis aborted, small ; palpi upturned, slender, the 2nd joint reaching to vertex of head and slightly fringed with hair in front, the 3rd long, porrect ; frons with small corneous plate below and larger corneous projection above ; eyes large



Figs. 1–6, frontal processes of *Diparopsis* : Fig. 1, *D. tephragramma* B.-Bkr. (ventral) ; fig. 2, *D. gossypioides* sp. n. (ventral) ; fig. 3, *D. castanea* Hmps. (ventral) ; fig. 4, *D. perditor* sp. n. (ventral) ; fig. 5, *D. perditor* sp. n. (lateral) ; fig. 6, *D. castanea* Hmps. (lateral).

round ; antennae of male bipectinate with moderate branches to apex, of female with short branches ; thorax clothed chiefly with scales, with the patagia very roughly scaled, the metathorax with spreading crest ; tibia moderately fringed with hair ; abdomen without crests. Forewing with the apex rounded, the termen evenly curved and hardly crenulate ; veins 3 and 5 from near angle of cell ; 6 from upper angle ; 9 from 10, anastomosing with 8 to form the areole ; 11 from cell. Hindwing with veins 3, 4 from angle of cell ; 5 from middle of discocellulars ; 6, 7 from upper angle ; 8 anastomosing with the cell to near middle.

Male genitalia ; harpes strongly asymmetrical ; right sacculus bearing a finger-like process.

Genotype : *Diparopsis castanea* Hampson.

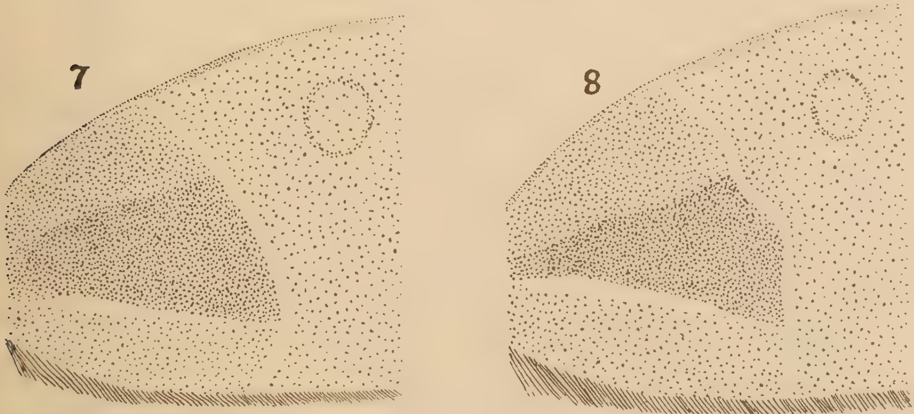
Key to the species of Diparopsis.

1. Frontal process without apical or ventral crater ; much flattened dorso-ventrally (figs. 4, 5)........*perditor* **sp. n.**
   Frontal process with crater ; little or not flattened dorso-ventrally........2
2. Frontal process with circular apex ; considerably broader than long (fig. 2)........*gossypioides* **sp. n.**
   Frontal process slightly flattened dorsally ; little or not broader than long......3
3. Frontal process with apical or sub-apical crater ; slightly longer than broad when viewed ventrally (fig. 1)........*tephragramma* B.-Bkr.
   Frontal process with ventral crater ; slightly broader than long when viewed ventrally (figs. 3, 6)........*castanea* Hmps.

Diparopsis castanea Hampson.

1902, *Ann. S. Afr. Mus.* **2,** p. 294.

♂. Head and thorax purplish pink mixed with whitish and some fuscous ; antennae ochreous white, pectus ochreous white, purplish pink in front ; abdomen ochreous white. Forewing purplish pink mixed with whitish, the costa irrorated with olive scales ; antemedial line whitish, oblique from costa to submedian fold, with slight indentation (fig. 8) above fold, then erect, a dark brown band before it from costa to median nervure and crossed by a wedge-shaped olive patch in submedian interspace ; an indistinct fine brownish medial line, oblique from costa to



Figs. 7–8.—Illustrating the course of the antemedial line on the forewing: Fig. 7, *D. perditor* sp. n. ; fig. 8, *D. castanea* Hmps.

upper angle of cell, then inwardly oblique ; a whitish postmedial line, excurved from costa to vein 4, then oblique, a similar subterminal line less incurved to costa, with blackish suffusion between them from vein 3 to inner margin ; cilia pale olive mixed with fuscous. Hindwing ochreous tinged with pinkish on terminal area ; cilia yellowish white ; the underside yellowish white, the costal and terminal area to vein 2 irrorated with rufous. Dorsal process of frons slightly flattened, with ventral crater, slightly broader than long when viewed ventrally.

Genitalia : right harpe tapering to a point with finger-like process arising before apex ; left harpe with two apical processes, the inner process stronger (fig. 9).

♀. Forewing pinker and less purple, the costal area not irrorated with olive, no band before costal half of antemedial line and the patch below the cell paler, the area between postmedial and subterminal lines on inner area and the cilia pale olive.

Expanse : 27–31 mm.

Food-plants : Cultivated cotton, also *Gossypium herbaceum*, var. *africana* Watt. and *Cienfugosia hildebrandtii* Garcke.

Mozambique, 1♂, 3♀♀ (including Type from Delagoa Bay) ; N.W. Rhodesia, 2♀♀ ; Natal, 1♂, 1♀ ; Nyasaland, 15♂♂, 11♀♀ ; Uganda, 1♀ (*H. H. King*). Type in British Museum (Natural History).

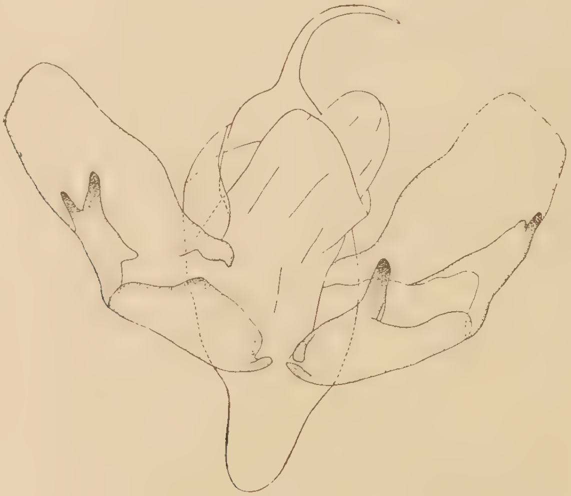


Fig. 9.—*D. castanea* Hmps., male genitalia.

Diparopsis perditor sp. n.

♂. Similar to *D. castanea* Hmps. but antemedial line without indentation (fig. 7) and frontal process much flattened dorso-ventrally and without ventral crater (figs. 4, 5).

Genitalia : right harpe very broad apically, without sub-apical process ; left harpe with two apical processes, the outer stronger (fig. 10).

♀. Similar to male. Expanse : 27–31 mm.

Food-plant : Cultivated cotton.

Sudan, 10♂♂, 16♀♀ ; Ethiopia, 2♂♂ (including Type from Meisso) ; Aden, 1♀ ; Nigeria, 4♀♀ ; Gold Coast, 1♀ ; Sierra Leone, 1♂ ; Senegal, 1♂, 1♀. Type in British Museum (Natural History).

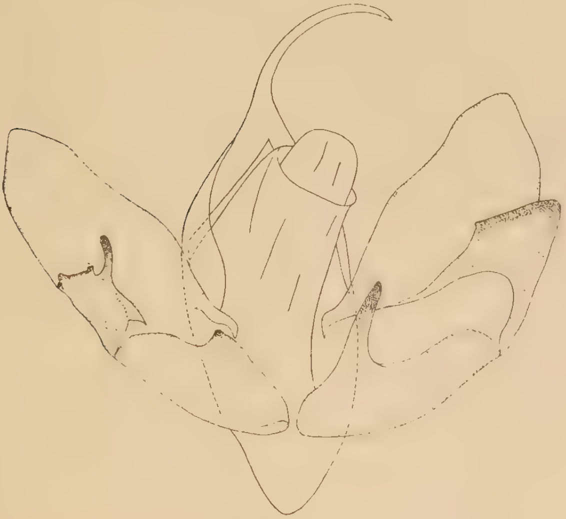


Fig. 10.—*D. perditor* sp. n., male genitalia.

Diparopsis gossypioides sp. n.

♂. Similar to *D. castanea* Hmps. but dorsal frontal process short, with blunt circular apex, of greater breadth than length (fig. 2).

Genitalia : right harpe gradually tapering apically, without sub-apical process ; left harpe with single apical process (fig. 11).

Food-plant : *Gossypioides kirkii* (Mast.).

Tanganyika : 1♂, north of Lindi, Type. Type in British Museum (Natural History).

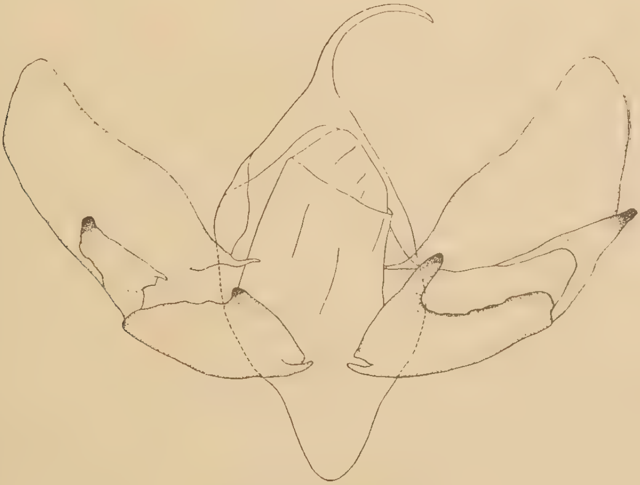


Fig. 11.—*D. gossypioides* sp. n., male genitalia.

Diparopsis tephragramma Bethune-Baker.

1911, *Ann. Mag. nat. Hist.* (8) **8,** p. 511.

♂. Head pale pinkish brown, thorax greyer, abdomen creamy. Primaries pale pinkish brown, with a wedge-shaped patch of darkish grey occupying most of the cell and part of the fold ; postmedian line pale, even, curved below the costa, then descending obliquely straight to the inner margin ; this line is preceded by an irregular dark grey line, and is followed by a broad oblique band of dark grey. Secondaries creamy white. Dorsal frontal process longer than broad when viewed ventrally, with apical, or sub-apical crater (fig. 1).

Genitalia : right harpe tapering to finger-like process, without sub-apical process ; left harpe with two apical processes (fig. 12).

♀. Like male. Expanse : 29–32 mm.

Food-plant : Unknown.

Angola : 1♂, Gunnal (Type) ; 1♂, N'Dallo Tando ; 1♀, Candy ; 1♀, Benguella. Type in British Museum (Natural History).

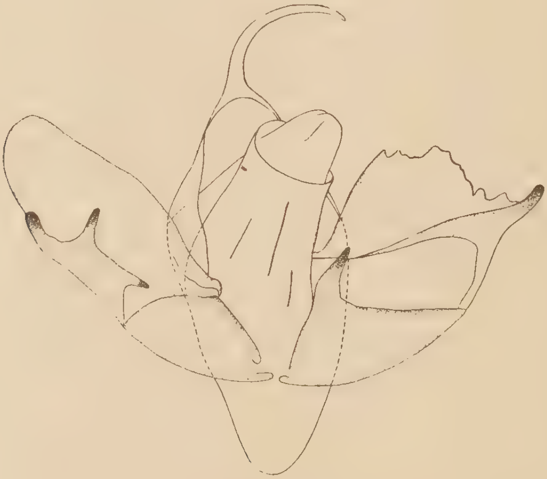


Fig. 12.—*D. tephragramma* B.-Bkr., male genitalia.

Acknowledgements.

I am greatly indebted to Mr. W. H. T. Tams, of the British Museum (Natural History), and to Dr. T. H. C. Taylor, of the Commonwealth Institute of Entomology, from both of whom I have received a great deal of help and many useful suggestions. I should also like to express my thanks to the Trustees of the British Museum (Natural History) for a grant which made this work possible.

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STUDIES ON BEETLES OF THE FAMILY PTINIDAE.*

VI.—THE BIOLOGY OF *PTINUS FUR* (L.) and *P. SEXPUNCTATUS* PANZER.

By R. W. Howe, B.Sc. and H. D. Burges.

(Plate XVI.)

Ptinus fur (L.) and *P. sexpunctatus* Panzer exhibit some marked differences in biology from the other warehouse Ptinids studied previously. They are similar to one another in morphology and they have been found in similar habitats, such as bees' nests.

The adult beetles of both species are described by Hinton (1941) and included in his key. The larva of *P. fur*, but not that of *P. sexpunctatus*, is included in a key to Ptinid larvae by Manton (1945). A modification of Manton's key to include the larva of *P. sexpunctatus* has been very kindly supplied by Dr. H. E. Hinton and is included here as an Appendix.

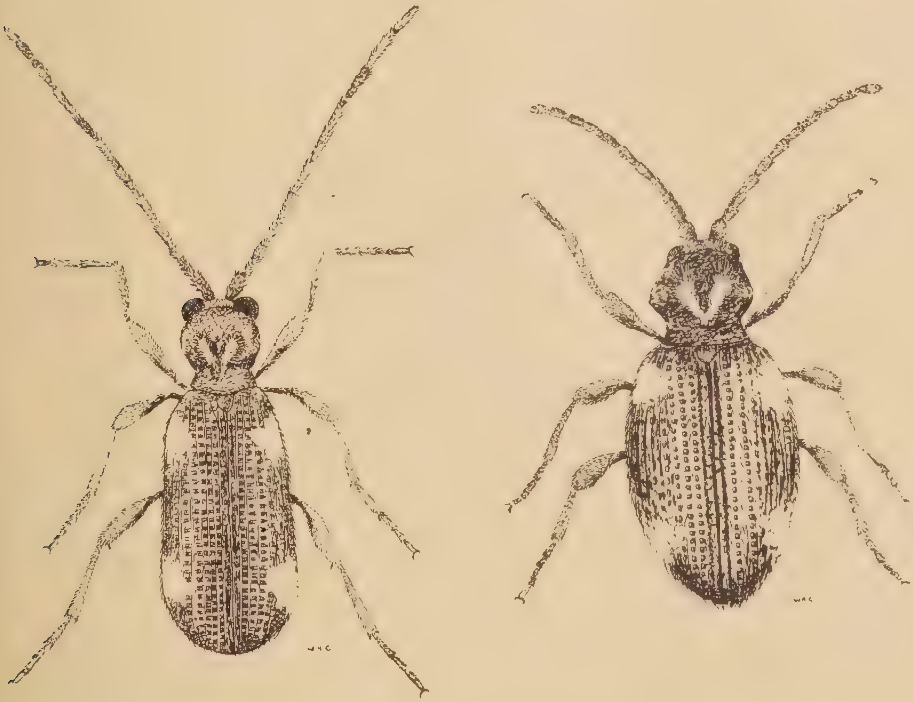

Fig. 1.—*Ptinus fur* (L.). Left, male ; right, female (×7).

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*Parts I, II and IV were published in Ent. mon. Mag., **85,** 1949, pp. 137–139, 189–190 ; **86,** 1950, pp. 325–326. Parts III and V were published in Bull. ent. Res., **41,** 1950, pp. 371–394 ; **42,** pp. 445–453.

Occurrence.

Ptinus fur (fig. 1) is cosmopolitan and an omnivorous feeder, occurring in small numbers in warehouses, stores, museums and houses. Hinton (1941) gives a list of foods on which it has been recorded including grain and grain products, dates, ginger, tobacco, dried plants, cocoa, insect specimens and bees' combs. Hinton & Greenslade (1943) found a larva on bird guano, and Frickhinger (1924) reported a bad outbreak in a house storing food in Hamburg. It is common in warehouses in the Soviet Union where it often damages wooden containers while building cocoons (Shapiro, 1948). It is sometimes found in bees' and wasps' nests. Linsley (1942) and Dobroscky (1925) found adults in a robin's nest.

P. sexpunctatus (fig. 2 & Pl. XVI) is less widely distributed than *P. fur* and is usually reported associated with nests of bees of the genera *Osmia* and *Megachile* and also with *Hoplitus adunca* (Panzer) and *Chelostoma nigricorne* (Nylander) (Linsley, 1942). It has been found in the nest of a house martin and in houses (Hinton, 1941). Howe (1949 and 1950) reported occurrences in middlings and in flour.

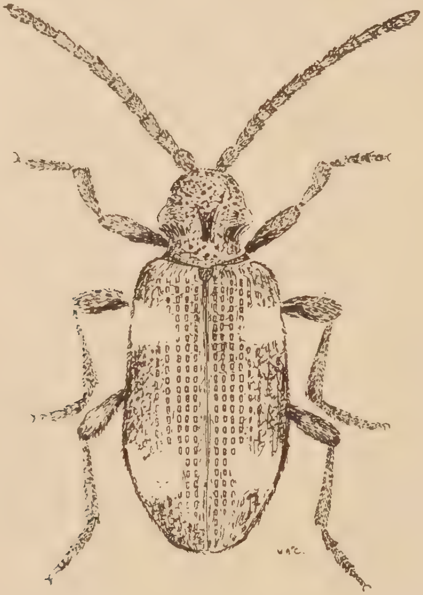

Fig. 2.—*Ptinus sexpunctatus* Panzer (×6·5).

Methods and Apparatus.

The method of rearing cultures is given by Howe (1949). Since both species multiply slowly at outdoor temperatures and a proportion undergo a resting stage, it is not easy to carry out laboratory experiments with them and the experiments described in this paper are incomplete.

The temperatures used were 30°±1°C. in a small incubator, and 25°±0·5°C., 23°±0·5°C., and 20°±0·5°C. in constant temperature rooms. All experiments were carried out at 70 per cent. relative humidity. At 30°C. and 20°C. this humidity was maintained in desiccators using a solution of caustic potash of specific gravity 1·238, but at 23°C. the air in the room was controlled at 70 per cent. R.H. The rooms had to be used also for field material which inevitably introduced psocids and mites when the experiments were prolonged. These contaminations were eradicated, as soon as they were noticed, by providing fresh tubes and food.

In each experiment on development, newly-hatched larvae were placed singly in $2 \times \frac{1}{2}$ inch glass specimen tubes. Food in excess of the amount needed for complete development was provided from the beginning, except in two experiments with *P. fur* at 23°C., one using unsieved fishmeal and the other a synthetic food (see below). In these the larvae were examined daily, so only a small amount of food was provided for the newly-hatched larva and the quantity increased later according to the size of the larva, usually after each moult.

The foods used were wheatfeed, fishmeal, and a synthetic foodstuff used by Fraenkel and Blewett (1943) consisting of 50 parts casein, 50 parts glucose, 5 parts yeast, 2 of mineral salts and 1 of cholesterol. For one experiment, the fishmeal was sieved and the fraction used that passed a mesh of 30 to the inch but was retained by 40. All foods were stored in thin layers in glass dishes in the experimental conditions of temperature and humidity for at least one week for moisture-conditioning.

The statistical methods used are from Fisher (1946) and Treloar (1939) and are given below :—

$$\text{Variance (V)} = \frac{\Sigma(x-\bar{x})^2}{n-1}$$ where $\bar{x}$ is the mean, x represents the observations and n is the number of observations

$$\text{Standard Error (S.E.)} = \sqrt{\frac{V}{n}}$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\text{S.E.}_1^2 + \text{S.E.}_2^2}} = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{V_1}{n_1} + \frac{V_2}{n_2}}}$$

where $\bar{x}_1$ and $\bar{x}_2$, S.E.$_1$ and S.E.$_2$, V_1 and V_2, n_1 and n_2 are the statistics of the two groups respectively. The table of t from Fisher (page 174) was used to obtain the probability (P) that the two groups originated from populations with the same mean.

Development of the Egg.

The data for the incubation period are given in Table II and are limited by the difficulty of obtaining enough eggs for experiment within a period of 24 hours or less. The eggs of *P. sexpunctatus* hatched at 30°C., but eggs of *P. fur* showed no signs of hatching at this temperature. For experiments on larval development at 30°C. eggs of both species were incubated at 23°C.

Development of the Larva.

The larval data are summarised in Tables I and II.

The duration of the individual larval instars of *P. fur* on fishmeal and synthetic food at 23°C. is given in Table I, but similar information is not available for *P. sexpunctatus*. The larvae of *P. fur* normally moulted three times but some had an extra moult. The number of insects showing this extra moult was increased by low humidity or poor food in the case of some other Ptinid species.

At 20°C. larval development is more rapid on wheatfeed than it is on fishmeal. As the same relationship applies at higher temperatures, development at 23°C. of

TABLE I.

Development period in days of larval instars of *P. fur* at 23°C. and 70 per cent. R.H.

Larval instar	Fishmeal		Synthetic food	
	N	M ± S.E.	N	M ± S.E.
I	27	13·7 ± 0·5	15	10·9 ± 0·2
II	26	16·4 ± 0·3	15	12·2 ± 0·1
Extra	9	22·8 ± 2·5	1	76
III Normal	14	32·1 ± 1·0	8	40·4 ± 3·8
III Diapausing	8	235·2 ± 21·3	3	248·7 ± 16·9

N = number of larvae completing the instar, M = mean, S.E. = standard error.

P. fur (non-diapausing individuals Table II) may be said to be quicker than at 30°C. or 20°C. The optimum temperature for rapid development is probably slightly above 23°C. as for *P. tectus* Boield. (Howe, 1943). The high mortality (Table II), the light weight of adults (p. 506) and the longer pupal period at 30°C. (Table II) support this estimate and suggest that 30°C. is near the maximum limit of development for *P. fur*. As the larval and pupal development of *P. sexpunctatus* was much quicker at 30°C. than at the lower temperatures used, the optimum temperature for rapid development is probably near 30°C. but, at this temperature, mortality was high.

The mature larvae spin strong cocoons in which to pupate and occasionally this made it impossible to delimit the pupal stage. At 20° and 23°C., some individuals of both species passed through a resting state, or diapause, as mature larvae in cocoons.

Diapause.

The diapause of *P. fur* larvae lasted about 220 days after normal larvae had pupated at 23°C. and about 280 days at 20°C. Examination of the data for the early instars of the diapausing and non-diapausing larvae gives some indication that, with fishmeal as food, the early instars of diapausing larvae developed a little more slowly than the other larvae. Similarly, pupae of diapaused larvae developed more slowly in four of the five experiments (*cf.* Table II). Statistical tests cannot be applied here as the numbers of insects used are small. Again, since the larvae were examined only once daily, there is a possible range of two days for each measurement, so the errors involved are as large as the differences recorded and statistical tests can hardly be valid. This point may, however, be worthy of closer examination in a study of diapause.

The data for *P. sexpunctatus* do not indicate a well defined diapause. As with *P. fur*, there was no larval diapause at 30°C. (Table II). At 20°C. on wheatfeed, one individual spent 156 days as a larva and another 280 days, the remainder developing to pupae in less than 100 days (Table II). Development was considerably slower on fishmeal at 20°C., three small groups developing in less than 190 days, 270 to 300 days and over 400 days respectively. Thirteen remaining larvae were still in diapause after 692 days. Five of these were kept at 25°C. and one adult eventually emerged after 727 days. All the remaining 12 finally died as larvae, the last after 910 days.

All the individuals of *P. fur* diapausing at 23°C. were male, and although only 14 adults were obtained in all, this seems very remarkable. Of the 13 larvae diapausing at 20°C. only four were male. The diapause of *P. sexpunctatus* seemed unrelated to sex.

TABLE II.

Development period in days at 70 per cent. R.H.

Temperature °C.	Food	Egg N	Egg M ± S.E.	Larva N	Larva M ± S.E.	Pupa N	Pupa M ± S.E.	Egg laid to adult N	Egg laid to adult M ± S.E.	Pre-emergence N	Pre-emergence M ± S.E.	Egg laid to emergence N	Egg laid to emergence M ± S.E.
P. FUR, NON-DIAPAUSING INDIVIDUALS.													
30	W.	no hatching†		13	88·5 ± 3·2	12	17·8 ± 0·5	—	— —	4	39·0 ± 5·5	—	— —
23	F.	—	— —	14	62·7 ± 1·8	13	16·0 ± 0·3	12	94·9 —*	12	37·2 ± 5·1	12	132·1 —*
23	Sieved F.	23	16·5 ± 0·2	23	67·3 ± 2·2	21	16·9 ± 0·2	17	98·8 ± 1·7	17	47·8 ± 3·0	17	146·7 ± 3·8
23	Synthetic	—	— —	8	63·2 ± 3·7	7	17·1 ± 0·5	7	96·8 —*	6	38·7 ± 7·0	5	130·3 —*
20	F.	43	19·7 ± 0·2	16	74·3 ± 2·3	15	18·5 ± 0·3	15	111·7 ± 2·5	15	48·7 ± 4·3	15	160·5 ± 3·8
20	W.	54	19·3 ± 0·1	24	62·8 ± 2·1	21	19·1 ± 0·2	21	101·5 ± 2·3	21	58·5 ± 2·0	23	156·6 ± 3·6
P. FUR, DIAPAUSING INDIVIDUALS.													
23	F.	—	— —	8	276·6 ± 21·1	6	17·5 ± 0·3	4	332·8 —*	4	45·8 ± 20·2	5	358·2 —*
23	Sieved F.	14	16·6 ± 0·2	14	317·2 ± 17·3	12	16·8 ± 0·3	9	339·3 ± 25·2	11	48·0 ± 3·2	10	381·4 ± 22·7
23	Synthetic	—	— —	3	272·0 ± 16·9	2	19·0 ± 1·0	1	315·6 —*	1	39·0 —	1	372·6 —*
20	F.	18	19·4 ± 0·2	11	355·6 ± 28·6	9	19·4 ± 0·4	9	356·2 ± 19·3	9	32·8 ± 4·8	9	389·0 ± 24·6
20	W.	4	19·0 ± 0·6	4	349·5 ± 38·6	3	22·7 ± 1·4	3	380·3 ± 50·9	3	44·0 ± 2·5	4	433·5 ± 37·2
P. SEXPUNCTATUS													
30	W.	11	8·2 ± 0·2†	8	35·3 ± 2·7	8	7·3 ± 0·4	8	50·7 —	8	71·4 ± 5·0	10	110 —
20	W.	50	17·1 ± 0·1	14 1 1	57·7 ± 1·2 156·2 280	16	16·2 ± 0·3	16	110·9 ± 15·1	15	103·0 ± 16·1	19	242·2 ± 30·9
20	F.	50	16·7 ± 0·1	5 5 2	142·2 ± 20·0 283·2 ± 2·8 408·0 ± 7·0	10	16·7 ± 0·5	11	274·8 ± 38·6	14	43·5 ± 7·1	17	325·9 ± 23·5

N = number of individuals completing the stage ; M = mean ; S.E. = standard error ; W. = wheatfeed ; F. = fishmeal.

Each period in a line of results refers to the same group of individuals, as far as they survived, except for periods marked* when the duration of the egg stage was taken from the results of parallel experiments shown in this table.

† = Eggs for experiments on larval development at 30°C. were incubated at 23°C., the 11 larvae of *P. sexpunctatus* which hatched at 30°C. not being used for experiment. The total development periods including the egg stage was obtained by adding the results for these two independent experiments and hence no standard error is obtainable.

Food.

Development of the larva was quicker on wheatfeed than on fishmeal (Table II) and the difference obtained for larval development at 20°C. is statistically significant ($P = < \cdot 01$). The synthetic foodstuff was also significantly better than fishmeal (Table I) for two instars ($P = < \cdot 01$). The slowness of the third instar on this artificial food suggests there was an insufficiency of food, or at least of one factor, although Fraenkel and Blewett (1943) found that the food was superior to wholemeal flour for *P. tectus*. Larvae of *P. sexpunctatus* developed more quickly on wheatfeed than on fishmeal at 20°C.

Mortality.

The larval mortality in the experiments performed at 20°C. and 30°C. was unsatisfactorily high but mortality at 23°C. was more reasonable (Table III). At 20°C., the experiments were prolonged by diapause and were affected by moulds, mites and psocids. Foods were therefore changed periodically, but the disturbance caused and the predation of mites undoubtedly contributed to the high mortality. The long diapause also contributes to the high mortality of *P. sexpunctatus* for only four of the 24 deaths on wheatfeed and 12 of the 27 deaths on fishmeal were recorded in the first 500 days of the experiment. At 30°C. the high mortality is probably a true result of an unfavourably high temperature. None of the larvae with prolonged periods of survival completed growth as indicated by the building of a cocoon so they cannot be considered to have entered diapause.

In all experiments some larvae had not spun cocoons at a date when growth should have been complete. At some later date shown by Table III all tubes were examined to discover whether larvae were alive or dead. Thus the numbers dying before diapause are exaggerated by this table. In general, little mortality during development was recorded except in the larval stage.

Development of the Pupa.

The pupal period for *P. fur* at 23°C. (Table II) was less than at either 20° or 30°C., which supports the suggestion that the optimum temperature for rapid development is near 23°C. The pupal development of *P. sexpunctatus*, like that of the larva (Table II), was much quicker at 30°C. than at the lower temperatures used. Only on sieved fishmeal (*P. fur*, 23°C.) was pupal mortality high—usually a sign of faulty cocoon building.

Maturing Adult.

The adult beetles of both species remained in their cocoons for a time (pre-emergence period) while their cuticles hardened and they attained sexual maturity.

The pre-emergence period of *P. fur* was much longer than that of any other Ptinid species studied. It was, however, shorter than the larval development period of non-diapausing insects, but more variable than any other single stage of development, except of course the diapausing larva. It was increased by temperatures of 20°C. or lower and varied with the food.

The life cycle of *P. sexpunctatus* is complicated by a second resting stage, as the developed adult remains inside the cocoon long after it has reached maturity. In culturing, it has proved advisable to break the cocoons and free the adults, which then behave and reproduce normally. It may be that under natural conditions they depend upon another agency breaking the cocoon, for in cultures, if left, many of the adults die in their cocoons. The weight of emerging adults did not appear to be affected by the periods of quiescence in the cocoon. On wheatfeed at both 20° and 30°C., the mean period spent by the adult in the cocoon was double the larval development period (Table II). On fishmeal at 20°C., the protracted larval develop-

TABLE III.

Mortality in the larval stage at 70 per cent. R.H.

Temperature °C.	Food	A days	B days	Larvae developed or dead in				Total No. larvae		Percentage larvae dead
				Less than B days		More than B days		used	dead	
				Developed*	Dead	Developed*	Dead			
P. FUR										
30	Wheatfeed	103	130	13	4	0	15	32	19	59·4
23	Fishmeal	80	100	14	7	9	0	30	7	23·3
23	Sieved fishmeal ...	105	120	24	1	15	2	42	3	7·1
23	Synthetic	80	100	8	1	4	0	13	1	7·7
20	Fishmeal	88	200	16	27	12	6	61	33	54·1
20	Wheatfeed	86	230	24	24	4	6	58	30	51·7
P. SEXPUNCTATUS										
30	Wheatfeed	46	140	10	22	—	—	32	22	68·8
20	Wheatfeed	70	150	19	2	4	22	47	24	51·1
20	Fishmeal	186	200	7	1	11	26	45	27	60·0

A represents the maximum period of development of a non-diapausing larva.
B represents approximately the first time a systematic search for dead larvae was made.
*These numbers do not always correspond with Table II since pupation could not be recorded when complete cocoons were made away from the tube wall.

ment was accompanied by a shorter period of adult quiescence in the cocoon of about the same length as for *P. fur*, which suggests that food may affect adult quiescence.

Female beetles of both species, on emergence from their cocoons, were heavier than males. The weights of adults from experiments at 20°C. are given in Table IV. Although development was slower, adult beetles bred on fishmeal were markedly heavier than those bred on wheatfeed, the differences at 20°C. being significant three times out of four. Adults of *P. fur* were lighter at the higher temperatures. For instance, the mean weight of males at 23°C. on fishmeal was 3·0 mg. and the mean at 30°C. on wheatfeed was 1·9 mg. These are both significantly different from the appropriate values in Table IV. Males of *P. sexpunctatus* were lighter but females heavier at 30°C. than at 20°C., neither difference, however, being statistically significant.

Mortality was usually small in the pre-emergence period, being more than one insect only in the experiment with *P. fur* at 30°C. in which seven adult beetles died in the cocoon.

Adult Life and Egg Output.

The adult beetles require food and lay their eggs over several months. The adult life and egg output has been investigated using single female beetles. They were kept, each with a male, in 2 × 1-inch specimen tubes containing food passed through a sieve of 60 meshes to the inch. Eggs were retained in the sieve when the food was sieved once or twice weekly. As adults can climb clean glass, each tube was closed with cotton cambric held in place by a cork through which a hole had been bored. The beetles need free water to drink for the maximum number of eggs to be laid. This was provided by placing them on moist cotton wool for about three hours after each sieving. With *P. fur* the sexes are distinct (fig. 1). There are no obvious external differences between the sexes of *P. sexpunctatus*, hence these adults were paired at random and only those laying eggs were retained. Two groups of beetles from different sources were used for each species.

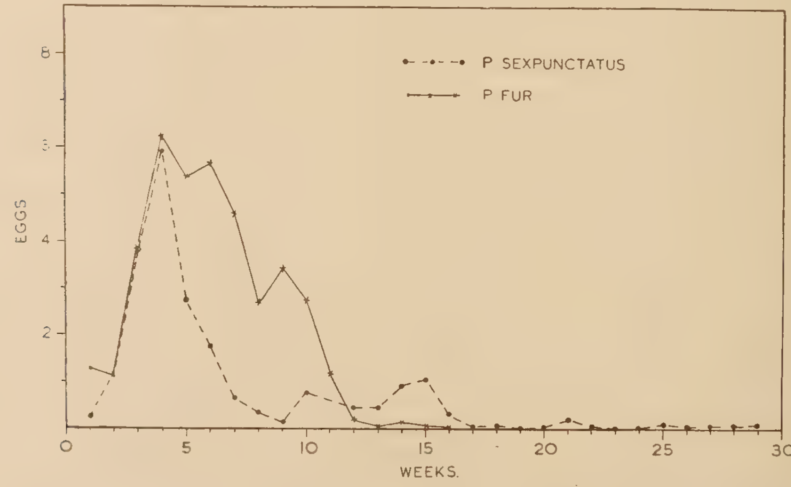

Fig. 3.—Mean weekly egg production of *P. fur* and *P. sexpunctatus*, based on 28 and 30 fertile females respectively.

Table V and fig. 3 summarise the results. On comparing the results for the two groups of beetles from different sources for both species, the only differences that are significant are those for the free adult life of *P. sexpunctatus* (Table V). There was a pre-oviposition period of one to two weeks after which egg output rapidly reached a peak, then fell more slowly as the females aged. The food was renewed at intervals,

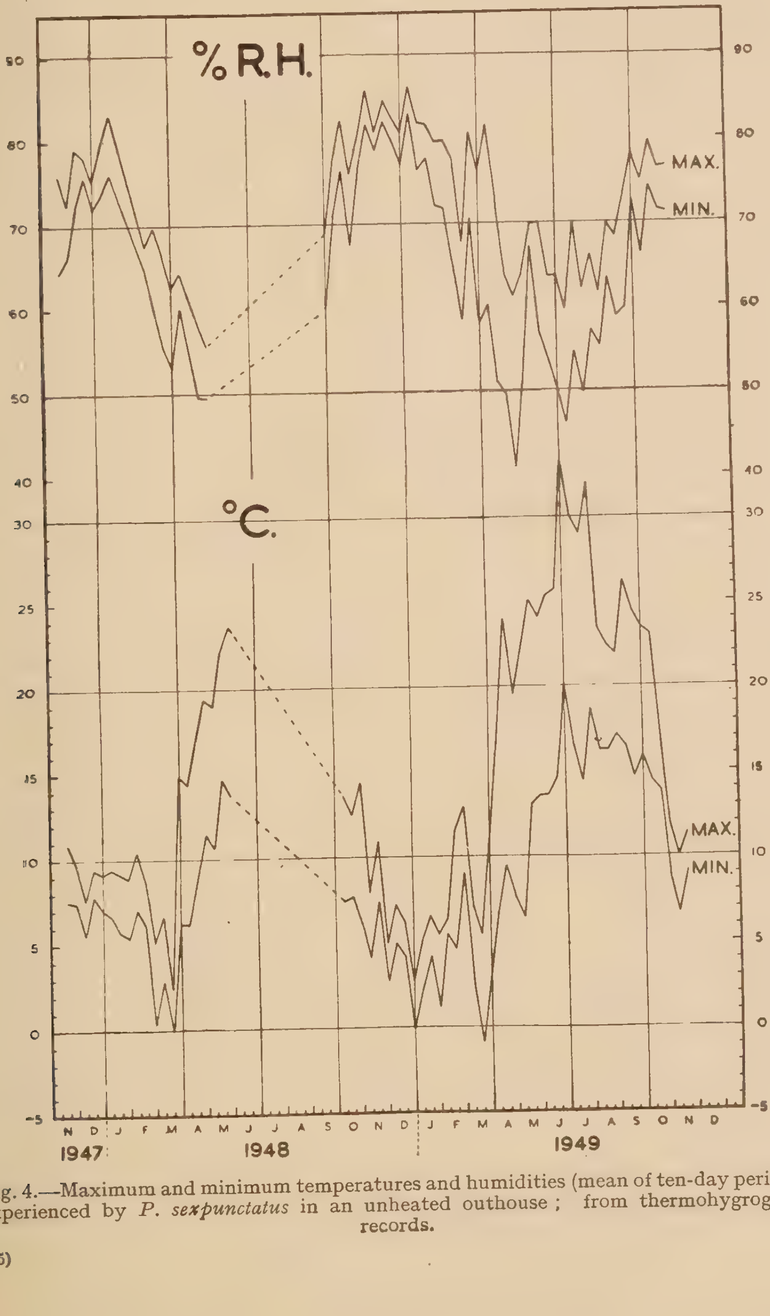

Fig. 4.—Maximum and minimum temperatures and humidities (mean of ten-day periods), experienced by *P. sexpunctatus* in an unheated outhouse ; from thermohygrograph records.

these changes having no effect on oviposition. Copulation probably occurs more than once during the oviposition period. The mean adult life of the male beetles was less than that of the females.

The adult life is short and egg output small compared with some other Ptinid beetles, especially *P. tectus* which has a mean total egg output of about 340 eggs under similar conditions.

Overwintering.

Thirty single larvae of *P. sexpunctatus* were placed in an unheated outbuilding in the late autumn of 1947. Fig. 4 gives the conditions experienced. One year later 13 per cent. were adults in cocoons, 27 per cent. were emerged adults, 10 per cent. large larvae and 50 per cent. had died. The following spring the remaining 13 per cent. adults in cocoons emerged, but there were still 10 per cent. (three) large larvae left. One larva became adult and emerged during the summer but there were still two larvae remaining in the autumn (*i.e.*, after two years). Thus overwintering can occur as young and mature larvae and as resting adults in cocoons. This is probably true also of *P. fur*, as larvae and adults of this species can be found in the field in all seasons of the year.

Natural Enemies.

Cotton and Good (1937) list four parasites of *P. fur*, *Cephalonomia xambeui* Gir., *Cryptus ptinivorus* Rond. (?), *Hecabolus sulcatus* Curt. and *Spathius exarator* (L.). *P. fur* has been recorded as the apparent host of *Lariophagus distinguendus* Foerster by Herold (1933) and Hüsing (1935). Hickin (1941) states that some cultures were attacked by the mites *Thyreophagus entomophagus* (Laboul.), *Glycyphagus domesticus* De Geer and *Hypoaspis* sp.

Discussion.

In comparison with the other warehouse Ptinids studied in this series, these two species are different in having a small egg output and a tendency to larval diapause at cool temperatures. The development period of *P. fur* is comparatively long. The survival and rapid development of *P. sexpunctatus* at 30°C. can be matched only by *Gibbium psylloides* Czemp. In addition, beetles of *P. sexpunctatus* show a period of adult quiescence before emerging from their cocoons, which is not found in the other species.

The data presented here correspond reasonably with the known field ecology of the two species. *P. fur* is an endemic species widespread in small numbers and rarely if ever numerous enough to be a serious pest. This is the situation to be expected in Britain from a species with a slow development rate and low egg production. *P. sexpunctatus* is a species normally associated with wild bees which has recently been found in warehouses. It could become quite a serious pest on certain foodstuffs as it develops quite quickly at 20°C. and very quickly at 30°C. and can overwinter in unheated conditions. It is hampered, however, by the apparent reluctance of the adult to bite its way out of its cocoon, resulting in a period of adult quiescence. It is possible that this is an adaptation to life in a bee's nest, and that it relies upon the bee to break the cocoon, so avoiding the possibility of emerging into a dormant nest.

Study of these species might be useful in work on diapause. There is some indication that poor foods and other conditions unfavourable to rapid development may be the cause of diapause in these species.

TABLE IV.

Weights in milligrams of adult beetles at emergence from their cocoons at 20°C. and 70 per cent. R.H.

	P. fur			*P. sexpunctatus*		
	♂	♀	Sign. of diff. between ♂ & ♀	♂	♀	Sign. of diff. between ♂ & ♀
Fishmeal	3·5	4·7	P<·01	4·0	4·5	P=·05
Wheatfeed	2·8	3·5	P<·01	3·7	4·0	P=·2
Significance of the difference between foods ...	P<·01	P<·01		P=·2	P=·02	

TABLE V.

Egg output of single female beetles and adult life at 25°C. and 70 per cent. R.H.

		P. fur		*P. sexpunctatus*	
	N	M± S.E.	N	M±S.E.	
Life of male beetles in weeks ...	23	12·1 ±0·5	8 19	*25·9±1·9 *10·3±0·8	P=<·01
Life of female beetles in weeks ...	24	14·0 ±0·7	8 20	*23·2±2·3 *16·7±0·6	P=·01
Egg output	27	38·6 ±6·3	30	21·3±3·5	
Number of females with egg output within the ranges 1– 20		10		22	
20– 60		13		6	
60–120		5		2	

N=number of individuals on which the results are based.

*The difference between the two groups is significant in this instance. In all other instances the groups are combined as the differences are not significant.

Summary.

The development of *Ptinus fur* (L.) and *P. sexpunctatus* Panzer at 70 per cent. R.H. and several temperatures has been studied.

The optimum temperature for rapid development of *P. fur* is about 23°C., at which temperature it completes its development in a mean period of 132·1 days on fishmeal. For *P. sexpunctatus* the optimum temperature for rapid development is slightly below 30°C., development being completed in approximately 111 days on wheatfeed at this temperature.

Eggs of *P. sexpunctatus*, but not those of *P. fur*, hatch at 30°C., which is near the maximum temperature for development of *P. fur*.

Larvae of *P. fur* normally moult three times at 23°C. on fishmeal, but some have an extra moult.

Well defined diapause as mature larvae in cocoons occurs at low temperatures in some individuals of *P. fur* ; at 23°C. this lasts about 220 days after normal larvae have pupated ; the period at 20°C. is about 280 days. Diapause at this stage also occurs in *P. sexpunctatus*. In experiments, the development periods of the diapausing and non-diapausing larvae overlapped to form a continuous series.

Some *P. sexpunctatus* pass through a period of quiescence as mature adults in cocoons ; at 23°C., this lasted 43·5 days on fishmeal and 103·0 days on wheatfeed (mean periods). In *P. fur* the pre-emergence period is 30 to 60 days, considerably longer than that of other warehouse Ptinids studied.

Of the foods, wheatfeed allows the quickest development for both species, but fishmeal produces the heaviest beetles.

The adult beetles live for several months. They require food and drinking water. Egg output is small, the mean number of eggs laid per female at 25°C. being 38·6 for *P. fur* and 21·3 for *P. sexpunctatus.*

P. sexpunctatus was able to overwinter in an unheated outbuilding and could possibly become a pest on some foods.

P. fur is unlikely to occur in large numbers, owing to its long development period, small egg output and tendency to larval diapause.

Acknowledgements.

The drawings were made by Mr. W. A. Carter and Mrs. W. Harding and the figures photographed by Mr. J. H. Hammond of this laboratory. Mr. Carter also carried out the experiments on egg output.

This work forms part of the research programme of the Pest Infestation Laboratory, and is published by permission of the Department of Scientific and Industrial Research.

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APPENDIX

Dr. H. E. Hinton provided us in 1945 with a modification of Miss Manton's key to include larvae of *Ptinus sexpunctatus*. Since this has not been published yet, we give below with his permission the changes he makes in the key. (Manton, 1945, p. 350.)

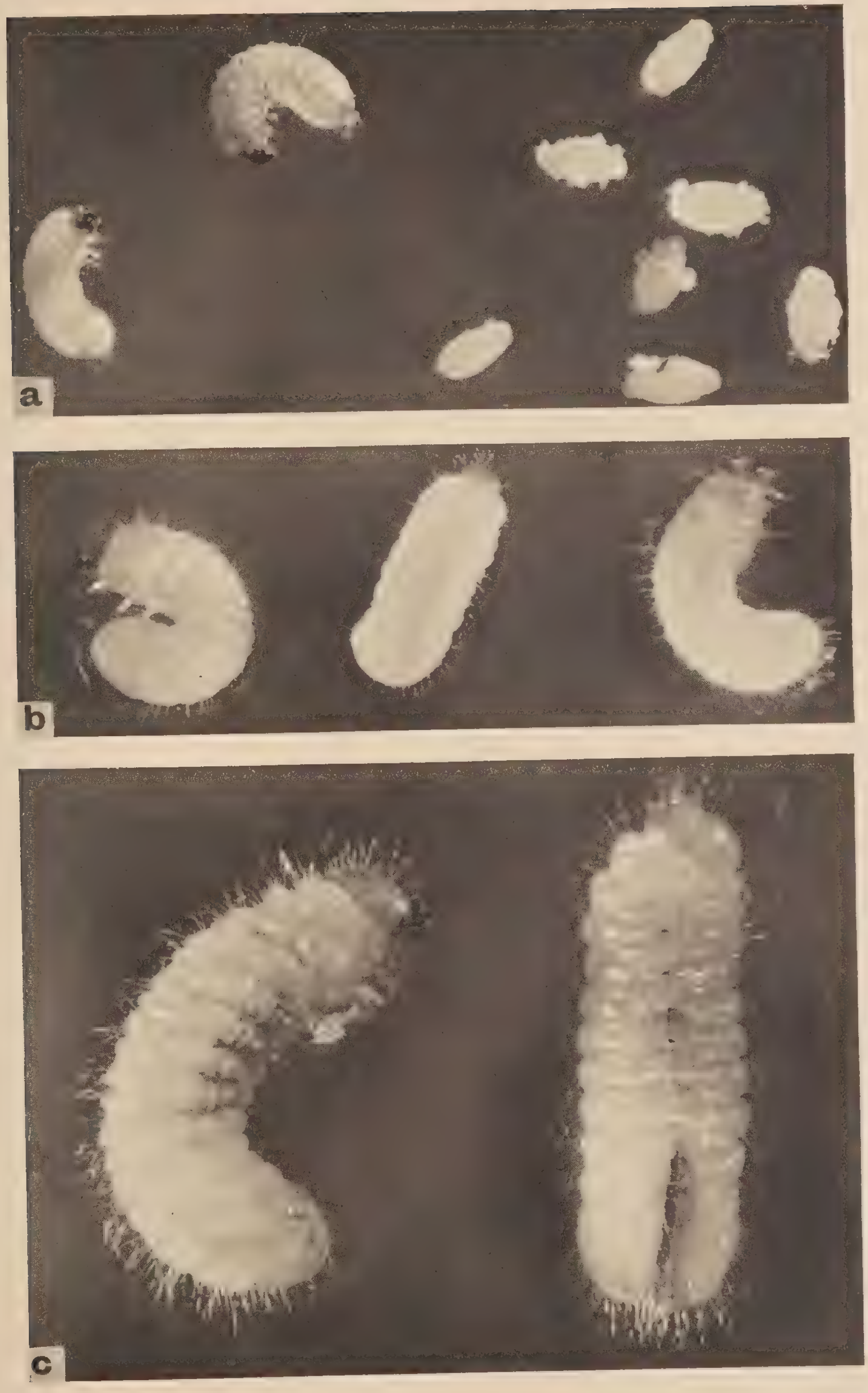
Paragraph 7, last line. For " 1½ " read " 2 ".

10. Epipharynx (Pl. V., figs. 40 & 41) with the setae of the diverging rows numbering about five ; and with setae lateral to the diverging rows absent. The upper side of the labium (Pl. VI., figs. 51 & 55) bearing no strong setae near and in addition to the pair near the middle line..10a

— Epipharynx (Pl. V., figs. 38 & 42) with the setae of the diverging rows numbering more than five ; and with setae lateral to the diverging rows present. The upper side of the labium (Pl. VI., figs. 48 & 52) bearing several strong setae near and in addition to the pair near the middle line..............................12

10a. Epipharynx (Pl. V., figs. 40 & 41) small, less than 130 μ wide at the transverse fold. Tooth on the mesal margin of the mandible obtuse. The upper side of the labium with 1–2 setae on each side across the base of the prementum ; and with about 1 pair of setae projecting beyond the anterior margin of the prementum between the palps..11

— Epipharynx larger, more than 160 μ wide at the tranverse fold. Tooth on the mesal margin of the mandible acute. The upper side of the labium with 4–5 setae on each side across the base of the prementum ; and with about 2 pairs of setae projecting beyond the anterior margin of the prementum between the palps..*Ptinus sexpunctatus*



Ptinus sexpunctatus, (*a*), eggs and first instar larvae (x 7 approx.); (*b*), older larvae (x 10); (*c*), mature larvae (x 10).

COMPARING THE EFFICIENCY OF INSECT TRAPS

By C. B. Williams, Sc.D.

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The problem of the best method for comparing the efficiency of different insect traps does not seem to have been well studied, although it is one of considerable interest to entomologists.

In our own work the question has recently arisen of comparing three different light-traps. It is not a simple problem, as not only are there differences between the traps which one wishes to measure, but no two traps can be on the same spot at the same time: thus the simple comparison is complicated by the effects of different locations and different nights.

Our previous work on trapping insects by light, carried out for over eight years, had shown (Williams, 1937 and 1940) that the difference between catches in different nights or in different traps are of a geometric nature, and not arithmetic or additative. Thus one trap catches on an average, say, *twice as many* insects per night as another trap—not 193 *more* insects per night.

Under these circumstances it is not correct to add catches obtained under different conditions and to use either the total or the arithmetic mean for the purpose of comparison. The correct comparison is the geometric mean, and the simplest way to get this is to convert the catch per night into a logarithm. The arithmetic mean of the logs. (obtained by adding the log. values together and dividing by the number of observations) is the log. of the geometric mean catch. For example, if catches of 100, 400, 50, 200, and 2,000 were obtained on successive nights, or in different traps, the logs. would be 2·00, 2·60, 1·70, 2·30, and 3·30; the mean log. would be 2·38; and therefore the geometric mean catch would be about 240 insects. The arithmetic mean of 550 insects is quite meaningless for comparison as it is completely dominated by the single large catch. Only one night had more than this number and four had less. The geometric mean is, on the other hand, the level above and below which one gets an approximately equal number of catches.

Thus when comparing two or more traps, allowances have to be made for location, for differences between nights or other successive periods, and for geometric changes in catch.

In its simplest form, the problem would be the comparison of two traps, and most of the difficulties would be overcome by having two locations (preferably not too different or too far apart) with the traps interchanged every night; so that in each pair of nights each trap would be once in each location. The catches are expressed in logs., and the mean log. catch in each trap after a series of repetitions would be the basis for a measure of trap differences.

Our immediate problem however concerned the comparison of three traps:—

(1) The Rothamsted Trap (see Williams, 1948), with a 200-watt ordinary electric light bulb.

(2) The same trap with a Phillip's 125-watt mercury-vapour bulb rich in ultra violet.

(3) A trap designed by H. S. and P. J. M. Robinson (1950) with a similar mercury-vapour lamp.

For completeness there should have been a fourth trap of Robinson's design with a 200-watt ordinary bulb, but unfortunately this was not available.

TABLE I.

Catch of Macrolepidoptera in each of the three light traps on 15 nights in August 1950, together with the position of the traps.

August	Trap 1		Trap 2		Trap 3	
	No.	Position	No.	Position	No.	Position
16	10	C	28	B	95	A
17	17	A	46	C	95	B
18	40	B	(100)*	A	205	C
19	20	C	87	B	137	A
20	24	A	247	C	351	B
21	27	B	195	A	1,396	C
22	18	C	216	B	482	A
23	42	A	356	C	323	B
24	35	B	148	A	421	C
25	43	C	115	B	312	A
26	13	A	157	C	156	B
27	24	B	11	A	253	C
28	31	C	66	B	109	A
30	10	A	188	C	220	B
31	15	B	22	A	671	C

*Estimated.

TABLE II.

Log, catch of Macrolepidoptera each night with three traps in three different positions changed each night : together with mean log. catch per night, mean log. catch for each trap in each position, for each trap in all three positions, and for each position with all three traps.

Position	Trap 1			Trap 2			Trap 3			Mean Log. per night
	A	B	C	A	B	C	A	B	C	
August										
16			1·00		1·45		1·98			1·48
17	1·23					1·66		1·98		1·62
18		1·60		(2·00)*					2·31	1·97
19			1·30		1·94		2·14			1·79
20	1·38					2·39		2·55		2·11
21		1·43		2·29					3·15	2·29
22			1·26		2·33		2·68			2·09
23	1·62					2·55		2·51		2·23
24		1·54		2·17					2·62	2·11
25			1·63		2·06		2·49			2·06
26	1·11					2·20		2·19		1·83
27		1·38		1·04					2·40	1·61
28			1·49		1·82		2·04			1·78
30	1·00					2·27		2·34		1·87
31		1·18		1·34					2·83	1·78
Mean Log.										All Traps
Pos. A	1·27			1·77			2·27			A. 1·77
Pos. B		1·43			1·92			2·31		B. 1·89
Pos. C			1·34			2·21			2·66	C. 2·07
All Pos.	Trap 1. 1·35			Trap 2. 1·97			Trap 3. 2·41			

General Mean Log. 1·91.

*Estimated value.

Three locations A, B and C about 100 yards apart were chosen in a small piece of woodland, of about 10 acres in all, on Rothamsted Experimental Station. The direct light of each trap was invisible from the others. The three traps were placed one at each point (3 at A, 2 at B and 1 at C) on the night of the 23rd to 24th August 1950. They were then kept in the woodland for 15 nights and each successive night moved round in the order A, B, C of the locations, so that by the end of the period each trap had been five times in each location. Unfortunately trap B failed on one night in position A. An estimated value has been inserted for this night for purposes of analysis.

Table I shows the actual catch of Macrolepidoptera each night in each trap together with the position in which it was working.

Table II shows the same data converted into a logarithmic scale, together with the mean log. for each night, and for each trap and each position.

TABLE III.

Analysis of variance from the log. catches of Macrolepidoptera.

	Degrees of Freedom	Total variance	Mean variance
Nights	14	2·4009	·1715
Position	2	·6840	·3420
Traps	2	8·5081	4·2540
Error	26	1·8248	·0702
Total ...	44	13·4178	

A statistical analysis of variance is shown in Table III, from which it will be seen that the total variance (*i.e.*, sum of squares of departure from the mean) for all the 45 trap-nights, is 13·4178; of this, 63·4 per cent. is accounted for by the differences between the traps, only 5·1 per cent. by the differences between the positions and 17·8 per cent. by the differences between the nights, leaving a small "error" of 13·6 per cent. of the total.

There is of course no doubt of the significance of any of the differences.

TABLE IV.

Geometric mean catches of Macrolepidoptera in 3 traps in 3 positions.

Position	Trap 1	Trap 2	Trap 3	All 3 traps in position
A	19	59	186	A=59
B	27	83	204	B=78
C	22	162	457	C=117
Trap in all 3 positions	22	93	257	

Mean all traps, all positions=81

Table IV shows the mean values at the bottom of Table II converted back to numbers. From the nine central figures in this table it is easy to see that there are considerable differences between the traps and between the localities. The highest mean catch (457 moths) is in Trap 3 when in position C; while the lowest (19) is with Trap 1 in position A.

The figures at the bottom of Tables II and IV give the mean catch for each trap in all three positions (an equal number of times in each), while the figures at the right are the mean catch in each locality (each trap represented an equal number of times).

It will be seen that the geometric mean catches per night for all Macrolepidoptera in the three different locations were 59, 78 and 117. It is curious to note that the poorest location was in a " riding ", while the best was almost completely covered and enclosed by trees and bushes. Ridings are usually considered by collectors to be more favourable locations.

The comparison of the traps showed that while the average night catch for all Macrolepidoptera in Trap 1 was 22 moths, the same trap with ultra-violet light captured 93 and Robinson's trap 257. This is a ratio of 10 : 42 : 117.

Similar analyses gave the following values for separate sections of the Lepidoptera :—

	Trap 1	Trap 2	Trap 3
Geometridae only	2·5 (10)	6 (24)	10 (40)
Amathes c-nigrum (L.) only	14 (10)	60 (43)	200 (143)
Noctuidae excluding *A. c-nigrum* (L.)	6·2 (10)	26 (42)	58 (94)

Thus the greatest relative difference is in the catches of *A. c-nigrum* (which was the dominant species and accounted for 5,976 out of a total of 7,242 Noctuidae) ; and the least difference was in the Geometridae, which were however not numerous and only 421 were captured in all three traps during the period.

The greatest numerical difference however appeared to be in the Noctuid moths generally designated by the name of " yellow underwings ", of which the catches in the three traps were as follows. Arithmetic totals are used here as there were too many days with zero catches to make the log. transformation reliable.

	Trap 1	Trap 2	Trap 3
Triphaena pronuba (L.)	2	112	211
Triphaena comes (Hb.)	0	22	24
Triphaena ianthina (Schiff.)	0	1	7
Lampra fimbriata (Schreber)	0	5	2
Total ...	2	140	244

Only one species showed a definite location difference, and this was the Noctuid moth *Cerapteryx graminis* (L.). Ninety-two individuals of this were caught, and all except two were in location "C" whatever trap was there. The numbers in the three traps were 8, 33 and 51. A somewhat similar distribution of this species was noted in July 1949 (French, 1951) when six Rothamsted traps were used for one month in the same woodland.

Summary.

An experiment is described in which three light traps were tested for relative efficiency by having three locations in a wood and moving the traps each day so that over a period of three nights each trap had been once in each position. The actual number of moths caught were converted to a logarithmic scale and the mean log.

(*i.e.*, log. of the geometric mean catch) was used for comparison. By analysis of variance it is possible to show what portion of the differences between the catches is due to the difference between the locations, to the difference between the nights, and the difference between the traps.

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STUDIES WITH SWEET CORN. THE FRIT FLY PROBLEM.

By Gordon HASKELL.

(PLATE XVII.)

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Crops of sweet corn (*Zea mays* L.) in England are subject to attacks by frit fly—*Oscinella frit* (L.). These attacks are often so devastating that a large proportion of the crop may be ruined. Female flies lay their eggs on germinating seedlings and grubs burrow their way through to growing points where they eventually pupate. Plants sustain all degrees of damage. Frit fly mostly attacks oats and, although Dawson (1945) and Haskell (1949*a*, 1949*b*) have referred to this pest on sweet corn, the only quantitative studies available are those of Cunliffe and Hodges (1946). They observed that different varieties varied in their resistance to attack. Absence of contributions by American workers is due to frit fly preferring wheat in the United States (Aldrich, 1920).

From 1946 to 1949 inclusive, field notes were made of frit fly damage to sweet corn crops grown experimentally at the John Innes former site in Merton, Surrey. In addition to summarising our present knowledge, publication of these will indicate some of the problems involved and also suggest lines of future research for controlling this pest which still makes sweet corn a risky crop in parts of southern England. Previous studies with sweet corn have been concerned with germination at low temperatures (Haskell, 1949*c*) and using ultrasonics to stimulate plant growth (Haskell and Selman, 1950).

Varietal Variation and Susceptibility.

To test whether there are differences between varieties in susceptibility to attack (*cf.* Cunliffe and Hodges, 1946), data were available from the 1948 varietal trials. Each of two randomized plots were sown with 80 open-pollinated and hybrid varieties introduced from the United States and Canada (Haskell, 1949). Except for three varieties with 20 seeds per row sown singly, 40 seeds of each variety were sown in pairs at 1 ft. intervals in rows 2 ft. apart. Sowings were made on 13th May and plants singled at the first leaf stage to 20 per row. Scoring for frit fly damage was made on 19th June, rows being judged according to amount of damage. The method of scoring is given in Table I and Plate XVII; all numbers in this paper refer to these assignation values unless otherwise stated.

TABLE I.

Method of scoring frit damage.

Description	Unattacked	Attacked but undamaged	Attacked with slight damage	Attacked with heavy damage	Very severely damaged or killed
Letter used in scoring ...	A	B	C	D	E
Assigned value for analysis	1	2	3	4	5
Transformation $\sqrt{x+0{\cdot}5}$	—	1·58	1·87	2·12	2·34

An analysis of variance was made on the 1948 data. The variance ratio for error mean square compared with varietal mean square was 1·3 which barely reaches the five per cent. point for significance, while the ratio for plot differences was not significant. The data were also re-analysed using the transformation for small numbers, $\sqrt{X+0{\cdot}5}$ and the same indications were again obtained. Perhaps the A–E scores are not sensitive enough fully to bring out observable differences in frit damage of different varieties.

The varieties listed in Table II were those more resistant ; these include both very early and very late types, suggesting that maturity* and resistance are not linked. This point is further tested later. The mean values for each plot were 4·38 and 4·20, indicating the generally low resistance of sweet corns to frit fly. The south plot was slightly less attacked. As the analyses indicated that there were no significant differences in damage in both plots, the interclass correlation coefficient was calculated for both plots for each of the 80 varieties. r was found to be $+0{\cdot}1324$ which probably could have been obtained by random sampling of an uncorrelated population. The amount of damage to a variety in one plot is not necessarily the same as in the other.

TABLE II.

Sweet corn varieties showing most resistance to frit fly in 1948.

	Variety	Value (Mean for 2 plots)
1.	Woodruff New Earligold Hybrid Woodruff Improved Sencross	3·0
2.	Connecticut Spancross (854·3 × 12) Tristate Iroquois Golden Robson Seneca Golden Hybrid Harris Northern Cross Ottawa Hybrid C H 1 Connecticut Spancross (C3·857–38 × 12) Henderson Cream of Gold Corneli Goldrush Hybrid Eastern States Sugar and Gold	3·5

80 varieties were included in the trial.

Data on the amount of frit damage in 1948 was also available from two plots of inbred lines of sweet corn. Again two seeds were sown per hole and spaced 2 × 1 ft. but only ten plants were grown in each row. These were sown on 13th May. Records are available for 58 inbreds. An analysis of variance was made on this data. The error mean square compared with " varieties " mean square gave a value of 2·3 for 57 degrees of freedom which is highly significant. Inbreds therefore differ in their susceptibility to frit fly damage. Inbred C.A. 13 showed greatest resistance (1·5 points) followed by C. 13–53 and inbred 2218 (2 points). A determination was made of the correlation coefficient for behaviour in both plots. Here, $r=+0{\cdot}4731$ which on testing gave $t_{[56]}=4{\cdot}02$. Hence the probability of obtaining such a value by random sampling of an uncorrelated population was small.

Both plots had, on the average, the same mean intensity of attack—3·24—which is somewhat lower than that for the two varietal plots sown on the same dates. As inbreds suffer from inbreeding depression, being less robust and slower growing than

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*Maturity was measured by the number of days lapsing between picking the first early varieties and picking all others. *Each row was picked once only*, when the majority of ears, after inspection, seemed ready for market. Each Maturity Value is the average of a single row in each of two separate plots (see also footnote to Table III).

open-pollinated and hybrid varieties, it is possible that slower growing strains are less subject to damage. Mean intensities of attack for 1948 varieties were therefore plotted against varietal earliness. No general trend could however, be detected. Hence actual maturity of a variety is no indication of its resistance. Table III shows that although there were a number of peaks of attack, and some earlier varieties were less damaged than later ones, on the whole earliness is no criterion of how much damage a particular variety will sustain. One point that still requires investigation is whether germination vigour is correlated with resistance.

TABLE III.

Maturity of sweet corn hybrids and frit attack (1948).

Maturity (in days) after 30th August	Number of varieties	Mean frit attack
0	2	4·00
4	2	2·50
5	2	1·95
7	7	4·14
11	5	4·20
14	2	4·75
15	9	4·27
18	7	3·63
21	2	4·50
22	4	4·50
25	7	4·21
27	1	4·00
28	10	4·45
29	1	4·50
39	2	4·25
40	2	3·75
50	1	4·00
51	10	4·35

All varieties were sown on 13th May and scored for frit damage 19th June.

The varieties whose ears were the earliest to ripen were picked on the 30th August. Maturity is measured from this date, *e.g.*, a variety picked five days after 30th August has a maturity of 5 in this experiment. Rows were picked once only, when the majority of ears appeared ready to send to market.

Another test was made on the data to see if varieties from different American breeders showed wide variation. Mean frit damage for varieties from six different sources was calculated (Table IV). The 1948 varietal trial had already shown (Haskell, 1949*b*) that, grown under our conditions, Harris, Robson and the Connecticut Experimental Station varieties produced plants with good characters, while some of those from the Central Farm, Ottawa, were poor and unsuited for English conditions.

TABLE IV.

Susceptibility to frit fly and source of sweet corn varieties (1948).

Source	No. of varieties	Mean Value
Harris, N. Y. ...	3	3·67 ± 0·28
Woodruff, Conn. ...	7	3·93 ± 0·73
Robson, N.Y. ...	7	4·07 ± 0·35
C.E.F. Ottawa ...	7	4·21 ± 0·39
Conn. A.E.S. ...	11	4·32 ± 0·56
Will, N.D.	5	4·60 ± 0·10

TABLE V.

Effect of sowing date on incidence of attack (1946).

Variety / Sowing date	Canada Gold	Elsom's Golden Sunshine	Extra Early Bantam	C 13 Inbred	Plumptip	Early White Market	Dependogold	Mean
(1) 1st March	40·0 (5)	50·0 (2)	27·3 (11)	— (0)	0·0 (5)	33·3 (3)	16·7 (24)	—
(2) 29th March	14·3 (28)	30·0 (20)	29·4 (34)	0·0 (9)	28·9 (38)	33·3 (18)	26·8 (41)	23·2
(3) 26th April	22·7 (22)	46·4 (28)	33·3 (30)	57·1 (7)	33·3 (38)	39·3 (38)	24·4 (41)	36·6
(4) 24th May	19·0 (21)	31·3 (16)	45·2 (31)	7·7 (13)	17·5 (40)	5·3 (19)	18·9 (37)	20·7
Mean for (2), (3) & (4)	18·7	35·9	36·0	21·7	26·6	26·0	23·4	

Figures refer to percentage of plants damaged by frit fly (scored 3rd July, 1946).
Numbers in brackets refer to total number of plants which germinated.

Yet the results from these sources given in Table IV indicate that frit attacks are not necessarily made on either less or more adaptable varieties. Other factors must be involved, as material from some breeders tended to be more susceptible than others.

Effect of Sowing Date.

There is evidence in oats that severity of attack varies according to when varieties are sown (Smith, 1931). Data are available from two experiments to test this with sweet corn. In 1946 a sowing date trial was conducted with one inbred and six hybrid varieties of sweet corn (Haskell, 1950). Four sowings were made at 28-day intervals from 1st March and a plus or minus scoring made for each plant individually. If it showed any frit damage a plant was scored (−) or if completely free (+). The A-E method of scoring was not used here. These data are set out in Table V for scorings made on 3rd July, calculated to show the mean percentage of plants damaged of the total number in each of two rows.

On the whole, frit fly attacked more plants from 26th April sowings, while there were considerably lessened attacks for 29th March and 24th May. The data for 1st March sowings are not too reliable, being based on low germination. The peak of attack thus appeared in 1946 at the end of April. Later sowings are therefore indicated to reduce damage. *Extra Early Bantam* and Elsom's *Golden Sunshine* were varieties more prone to attack during the sowing season between 29th March and 24th May, while *Dependogold* and *Canada Gold* were less attacked. It is possible that the sowing dates chosen did not coincide with breeding fluctuations of the frit flies.

Peaks of attack probably vary from year to year. In 1949 a sowing date trial of the three most important sweet corns for English conditions, *viz.*, *John Innes Hybrids I and II* and *Northern Cross* was held at Merton. Four fortnightly sowings were made from 1st April to 13th May inclusive. There were two randomized plots of 50 seeds later thinned to a maximum of 25 plants. The rows considered as a whole, were scored A to F (where F=killed plants and has assignation value=6) for frit damage on 23rd June. An analysis of variance based on the assignation numbers is shown in Table VI. Here there are significant results for different varieties and sowing dates, but there are no plot differences, nor are there any significant values for interactions. Least attacks on these three varieties were for 13th May sowings. This again confirms the value of comparatively later sowings for reducing frit incidence. This is no disadvantage as Haskell (1950) has shown that there is no gain by sowing sweet corn too early.

TABLE VI.

Analysis of variance of 1949 sowing date trial involving 3 hybrids.

Item	S.S.	N.	M.S.	V.R.	t.	P.
Varieties (V)	6·58	2	3·29	6·77		·05
Sowing dates (S.D.) ...	30·79	3	10·26	20·69		·01
Plots (P)	1·04	1	1·04		1·46	N.S.
V–S.D.	3·08	6	0·51	1·06		N.S.
V–P	0·08	2	0·04	11·66		N.S.
S.D.-P	1·46	3	0·50	1·02		N.S.
Error	2·92	6	0·49			
Total ...	45·96	23				

Figures refer to assignation values.

Wind Direction and Spacing.

Aerial studies with frit flies have shown that they can be obtained at various heights up to 60 ft. with a maximum at 16 ft. after which numbers drop off, although they are still plentiful between 33 ft. and 60 ft. (Riggert in Wolfenbarger, 1946). They were found to be dispersed more abundantly near to the ground, their numbers decreasing with height above ground (Korting in Wolfenbarger, 1946). An analysis to test whether there was lateral distribution from a source was made on data from an experiment designed in 1949 to study wind pollination along stringers from a central mass of pollen-bearing plants of the same sweet corn hybrid placed according to the points of the compass (Bateman, 1948). Assignment figures were given to individual plants. The hybrid variety used was *Seneca 60* (Hart and Vick), and as all plants were genetically of similar constitution there is here an opportunity of examining environmental factors influencing attack, as intra-variation is thus reduced.

TABLE VII.

Analysis of variance of plants in central mass (1949).

Item	S.S.	N.	M.S.	V.R.	P
Rows running in E–W direction	4·51	10	0·45	2·87	$<0{\cdot}05$
Rows running in N–S direction	12·88	10	1·29	1·00	N.S.
Error	129·31	100	1·29		
Total ...	146·69	120			

Figures refer to assignation values

Considering first the central square mass where plants were spaced at 1 ft. apart with 11 plants in the rows, the analysis of variance (Table VII) shows that the value for row plantings in the E–W direction is barely significant whilst that for row plantings running N–S is not significant. In the stringers the mean frit attacks according to direction and distance from the central mass are given in Table VIII, for readings made 11th July 1949. Here there were 4 ft. between plants along stringers which commenced 4 ft. from the edges of the central mass. The N. stringer received considerably less damage than any of the others, while the S.E. and S. stringers were severely damaged. The prevailing wind direction for the first ten days of July 1949 was variable : N.E.3 : N.W.3 : S.E.2 : S. and W. 1, but on the whole the prevailing wind blew on to the central mass from the north and this could perhaps be associated with the lower incidence of frit in the northern stringers.

TABLE VIII.

Analysis of variance of stringers (1949).

Item	S.S.	N.	M.S.	V.R.	P.
Directions	41·17	7	5·88	3·00	N.S.
Distance from central mass	7·34	10	0·73	2·68	N.S.
Error	137·21	70	1·96		
Total	185·72	87			

Figures refer to assignation values.

The data from the stringers were also analysed to determine whether attack along stringers is associated with proximity of stringer plants to the central mass which might act as focal point of infection. The analysis of variance set out in Table VIII indicates that neither direction from the central mass nor distance from it reaches significance level. This confirms previous experience that frit flies are scattered at random throughout the fields and attack plants locally. In other words, they do not concentrate at selected points within the crop.

Discussion.

Although sweet corn in its evolution in an American habitat has not been subject to infestations of frit fly, different varieties already show differences in susceptibility to this pest ; this is quite pronounced among inbred lines. As it is known that there are genetical bases for resistance and susceptibility of maize to aphids (Walter & Brunson, 1946), the European corn borer (Patch & Everly, 1948) and other insect pests, it is suggested that probably the basis for resistance to frit fly is of a genetical nature. It would be of polygenic form as all grades of resistance occur. Selection for increased resistance should, therefore, be possible as has been carried out already for locust-resisting types of maize in Argentina. The list of more resistant hybrids given in Table II contains several, such as *Northern Cross*, which are highly suited for English conditions compared with varieties like the *John Innes Hybrids*, now widely grown throughout England, which are rather susceptible to frit attacks.

The frit fly in evolution has adapted itself to live on cereals but the factors that influence its attraction, not only to sweet corn plants generally but also to some varieties more than others, are still unknown. Neither earliness nor lateness of varieties are influencing factors but further attention could be paid to see whether varieties, which easily produce tillers after their leading stems are killed, are more suitable for frit infested areas.

Several means can be suggested for controlling frit fly attacks on sweet corn.

(1) Crops should be grown preferably in areas which are known to be frit free, from experience with local oat crops. (2) It may be possible to reduce incidence of attack by timing the vulnerable, *i.e.*, germination, period with sowings made in the trough of frit breeding periods, indicated above as being towards the end of May in Merton. According to Smith (1931), there are probably three main generations a year. (3) Prophylactic methods would include seed and soil treatment, such as—dusting the seed rows with DDT or sowing oats with the corn, in the hope that the frit flies would prefer the oats. (4) Genetical methods would include the use of varieties that quickly produce stout tillers replacing the main stem after the growing points have been eaten away by grubs ; the use of less susceptible varieties and selection from these of resistant strains ; using a locust-resistant maize from Argentina and back-crossing this into sweet corn. This assumes that the plant chemical repellent to locusts will also repel frit flies. Unfortunately there are as yet no locally selected strains of sweet corn in different parts of England with which new American material can be inter-bred to bring in resistance. However, the use of sturdy hybrids, such as *Northern Cross*, sown at times suitable for the particular area and knowledge of what to expect from frit fly in that area should help considerably to overcome this pest at present.

Summary.

All varieties of sweet corn, in the seedling stage, are susceptible to frit fly attacks in England.

Different open-pollinated, hybrid and inbred varieties differ in susceptibility to damage by frit, indicating a possible genetical basis for resistance. Some breeders have produced less susceptible types than others.

Susceptibility is not associated with adaptability or maturity (*i.e.*, comparative earliness when whole rows were ready for picking) of varieties but there is indication that seed origin is of some importance.

Sowing sweet corn at different dates influences the severity of attack. Peaks vary according to variety and year but generally sowings late in May are preferable.

Distribution of attack on a crop is at random, although there is a suggestion that plants in stringers from a central mass are more damaged in the direction of the prevailing wind but there is no regular decrease in incidence with distance from the central mass.

Methods of tackling the frit fly problem include growing sweet corn in frit free areas, timing sowing dates, using prophylactic measures and breeding more resistant strains on genetical principles.

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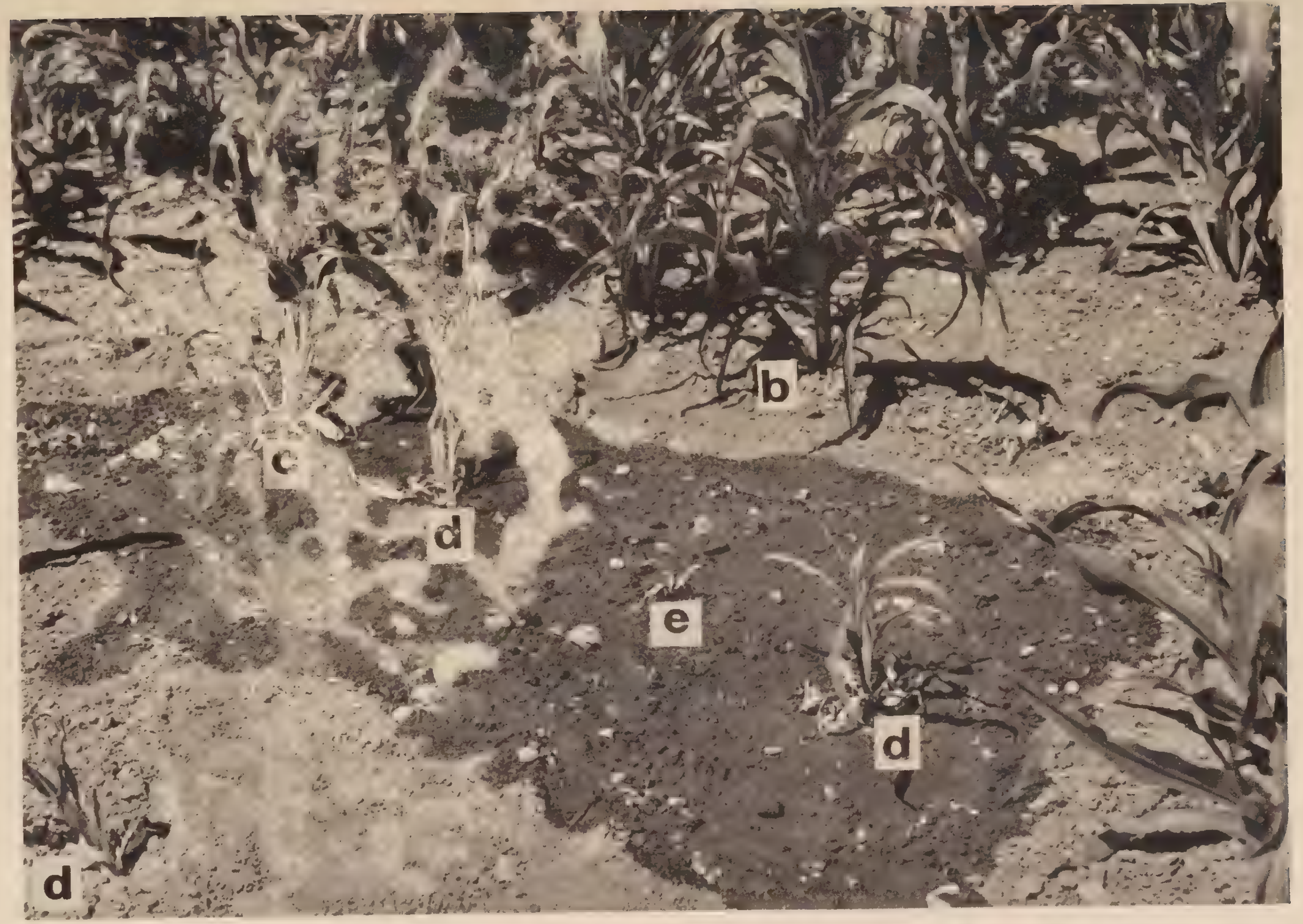
PLATE XVII.



Part of the Sweet Corn sowing date trial in 1949 at Merton, Surrey, showing all stages of damage by frit fly. The row nearest to the camera has been watered to make the plants stand out. This row comprises John Innes Hybrid No. 2 sown on April 1st. 1949. Grades c, d and e can be seen in this row. Behind are John Innes No. 1 plants of grade b sown on 13th May. Both differences in varieties and sowing dates influence amount of damage.

AN ECOLOGICAL STUDY OF MOSQUITOS IN WUHAN AREA.

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Very little work of any kind has been carried out on mosquitos in Wuhan, which is situated in the heart of the vast area of central China, except for a brief survey made by Dr. Feng Lan-chou in 1932. The results of collections made in August and September of that year were presented in his paper (1933) and included the following species : *Anopheles hyrcanus* var. *sinensis* (Wied.) ; *Armigeres obturbans* (Wlk.) ; *Mansonia uniformis* (Theo.) ; *Aëdes albopictus* (Skuse) ; *Culex fuscanus* Wied. ; *Culex tritaeniorhynchus* Giles ; *Culex pipiens* L. ; *Culex sinensis* Theo., and *Culex bitaeniorhynchus* Giles.

The present writers found all of the above species except the last two but, in addition, found *Culex fatigans* Wied. and *Culex vagans* Wied.

Methods.

This study was carried out between July and December 1947. Collections were made at Yu Kian Wan (the Hupeh Provincial Agricultural College), Lo Chia Shan (Wuhan University), Wuchang and its vicinity, Hanyang and its vicinity and in the Hankow area.

Adult mosquitos were usually found in secluded corners inside or underneath buildings, sometimes on window screens and in toilets, soldiers' dormitories and in poorer class dwellings and animal sheds. Larvae, according to species, occurred in the following breeding places : *Armigeres obturbans* almost exclusively in toilet jars diluted with rain water ; *Aëdes albopictus* in stagnant water in any artificial container ; *Anopheles hyrcanus* var. *sinensis* and the other species recorded, in rice fields, ponds, pools and pits.

In collecting adults, a cyanide vial, one inch in diameter, was used very successfully when free from moisture. The insects were captured while biting, or resting on an object, by slowly moving the killing vial to within an inch or so and then quickly covering them. When they were to be kept alive for egg-laying or dissection, the insects were captured in a similar manner with a clean vial.

In order to collect adults out of doors at night, a black cloth trap 12×8×8 in. was employed during the summer. The trap was designed so that a mosquito entering to shelter in darkness could not readily escape after daybreak. The trap was finally closed by quickly covering the opening and buttoning it up. The specimens so captured were subsequently killed by exposing the trap to the noon sunlight.

For collection of larvae, since a white-enamelled dipper as commonly used was not available, a dip-net covered with fine mesh linen was employed. A large-mouthed pipette served to collect and transfer the specimens which were finally preserved in 75 per cent. alcohol.

For microscopic examination the whole larva was mounted on a slide after about 20 hours' soaking in alco-glycerol, a mixture of equal parts of 70 per cent. alcohol and pure glycerin. During soaking, alcohol escapes gradually on evaporation, while penetration of the tissues by glycerin goes on until the specimen becomes thoroughly transparent. Balsam mounts of whole larvae were sometimes required for taxonomical study.

Topographical Notes on Wuhan.

The following brief account of the topography of the area is given to indicate the environment in which the mosquito species occur.

Wuhan area, the delta of the Han River (30′ 50′ N., 114′ 20′ E.) lies in the centre of the alluvial plain of the middle Yangtze, just on the border between the Palaearctic and Oriental regions. Elevation is about 150 feet above sea level, with no mountains worthy of the name within a hundred miles. There are, however, a number of low ranges of hills, rising rather abruptly out of the surrounding plain to an altitude of 200 to 300 feet, and running roughly east and west. With the exception of those named Chiu Feng and Lo Chia, the hills in the vicinity of Wuhan are practically treeless.

These ranges are composed mainly of sandstone, including quartzite, micacious sandstone (Hong Hill) and massive sandstone (Tortoise Hill). Conglomerate is generally found in conjunction with the sandstone. Beds of shale of varying thickness are to be found (underlying the series) in most places. By far the greater part of the area is, of course, flat alluvial plain with considerable areas of " red beds ", deposits of eroded red sandstone from Szechwan.

There are some well known lakes in this region known as Tong Hu (surrounding the north-eastern side of Lo Chia Hill), Sah Hu (west of the former), Chiang Hu (within Wuchang city), Nan Hu (south of Wuchang) and Yuan Ya Hu (north-west of Hanyang city).

With the exception of Tong Hu (East Lake), in which no aquatic stage of mosquito was found, the borders of the lakes, if decaying vegetation or other organic matter is present, usually favour the development of *Culex* spp. Lotus ponds with floating scum also serve as *Culex* breeding places. Anopheline larvae are almost exclusively rice field breeders ; none was found in any hill stream except on Chiu Feng, where Dr. M. K. Hu collected *A. hyrcanus* var. *sinensis* during a brief visit to Wuhan.

During the rainy season (June and July) many temporary pools are naturally made ; they do not generally help to increase the mosquito population unless they are polluted by being near human habitation.

Seasonal Incidence of Mosquito Species.

Judging from observations during the collecting period, the incidence of the different species varies markedly with seasonal changes. Thus, when collection began at the end of June 1947 two domestic species, *Culex fatigans* and *Armigeres obturbans*, were found to be abundant. The former became less abundant towards the hottest part of the year (July and August), whilst the latter, on the contrary, throve all through the hot summer and early autumn, and finally became scarce when the temperature dropped to freezing point. *Mansonia uniformis* and *Aëdes albopictus* were plentiful during August and September. By October *Culex tritaeniorhynchus* was breeding extensively in some of the bomb craters near Sah Hu, north-east of Wuchang. As winter began, the activity of *Culex pipiens* was considerable. Adults of this species survived even at freezing point, and a few could still be found flying indoors at the beginning of spring. They became active and began to bite humans in the early part of March, when the room temperature was 70°F.

The breeding of Anopheline mosquitos was most extensive during August, September and October. This coincides with the period of lowest mean relative humidity (69·1, 65·6, 63·9 per cent. respectively), or it may be that relative humidity affects the survival of *Anopheles* (see Table I). It should be noted that the optimum humidity for survival of *Anopheles* for the Canton region was the lowest, namely 67·7 per cent.

Bionomics of the Species Studied.

The following account of observations both in the laboratory and in the field includes notes on bionomics and relation to human pathology. Ecological conditions in breeding sites are discussed in a later section.

Anopheles hyrcanus var. *sinensis* Wiedemann, 1828.

Adult females freely attack man as well as animals such as cattle. Blood-fed females were collected in abundance in soldiers' dormitories at Yu-Chia-Wan. Five blood-filled females from near Wuchang were kept for egg laying in a cage containing a water-filled dish. More than 40 eggs had been laid by next morning and were floating on the water surface, arranged end to end in connected triangles. After two days' incubation (average noon temperature 95°F.) all hatched, but unfortunately owing to a sudden drop in temperature they died, and no record of the life cycle was obtained. Li and Wu of the Entomological Bureau of Hangchow reported (1934), as a result of their work in Hangchow, that this required 11 days for completion.

The preferred breeding sites of this species are chiefly rice fields, pool margins, and slowly running streams.

Monthly figures for climatic factors, and the incidence of malaria and of *Anopheles hyrcanus* var. *sinensis* are shown in Table I.

TABLE I.

Rainfall, temperature and relative humidity in relation to* incidence of malaria and abundance of *Anopheles* in Wuhan.

Month	Mean rainfall (ins.)	Mean temperature F.	Mean rel. humidity (per cent.)	Malaria incidence (per cent.)	Abundance of *A. hyrcanus sinensis*	
					Adult	Larva
June ...	9·6	79·7	79·7	15	(collecting not started)	
July ...	7·1	85·5	71·0	15	40	33
Aug. ...	3·8	85·5	69·1	18	96	55
Sept. ...	2·8	72·9	65·6	24	123	84
Oct. ...	3·2	62·1	63·9	19	85	40
Nov. ...	1·9	54·0	71·6	7	15	8
Dec. ...	1·1	38·9	73·3	·5	0	0

* Rainfall, temperature and relative humidity are averages for ten-year period 1916–1925 obtained from *Hankow Weather Guide*. Data on malaria incidence were obtained from three hospitals ; St. Joseph's, Church General and London Mission, at Wuchang.

It will be observed that *A. hyrcanus* var. *sinensis* is most abundant during the months of August to October, with a peak in September two to three months after the peak of rainfall in June, a period when there is still plenty of water, but with little danger of larvae being washed away by heavy rains. The relative humidity during this period is at its lowest for the year, ranging from 69·1 to 63·9 per cent. By December, when the mean temperature has fallen below 40°F., all activity has ceased.

The species was most abundant when incidence of malaria was at its height, and as no other species of *Anopheles* was collected there is good reason to believe that this species is the vector of malaria in this area.

The combined records of the three hospitals for 1947 (1,937 patients) included 232 malaria cases, of which 31 gave positive blood tests. Statistics showed *Plasmodium vivax* 7 per cent., *P. falciparum* 4 per cent., and *P. malariae* 1 per cent.

The species is also an important vector of filariasis in China. There was only one case of elephantiasis in the records for 1947 at St. Joseph's Hospital, Wuchang, and no parasite was found in the test.

Mansonia (*Mansonioides*) *uniformis* (Theobald), 1901.

Observations indicated that females of this species were entirely anthropophilic in their feeding habits. Because of their persistence when biting, it was possible to capture a specimen immediately it started to bite. They were greatly attracted to light at night. Considerable numbers were collected during August at Lo Chia Shan by first attracting them into the house by means of a light and then catching them with the killing bottle. The females bite man in the open air as well as indoors. They are especially active during the night in the rainy season. Feng (1938) has recorded that heavy rain will not prevent them flying. During the hottest months of July and August 1947 they were found in abundance in bushes and weeds throughout the region. The hospitals of this area had no medical information on this species.

Armigeres obturbans (Walker), 1860.

This is a very common domestic species throughout the region, and may be found by day in large numbers both in toilets and dark interiors. The females actively attack humans, feeding at night but, if the house is dark, they may attack at any time.

The most favourable breeding site was found to be a toilet jar containing human faeces and urine, diluted with rain water. Larvae were also found in abundance in garden compost pits containing very foul water.

No information regarding this species as a possible vector of disease is available for this region.

Aëdes (*Stegomyia*) *albopictus* (Skuse), 1895.

This species is a vigorous daytime biter, but it does not occur in large numbers. Females are often to be found by day amongst bushes and weeds around human habitations. The preferred breeding site is in collections of rain water, especially in household containers out of doors. A considerable number of *Aëdes* larvae was found in earthenware vessels stored in an open yard beside a pottery shop in Wuchang.

The life cycle of this species was observed in the laboratory by rearing 28 larvae hatched from eggs freshly deposited by a captured female. The water used for rearing was brought from the breeding site mentioned above. The average temperature during the breeding period was 77·7°F. room temperature, and 76·5°F. water temperature. The results obtained were as follows : Incubation period two days ; larva eight days ; pupa two days ; period of emergence one day ; total, egg to adult, 12–13 days.

The larva of this species is not so susceptible to unfavourable rearing conditions as that of others and this makes it a suitable subject for laboratory study.

It has been shown by Simmons, St. John and Reynolds (1930) that *Aëdes albopictus* is a very important vector of dengue fever in the Philippines.

In China, no experimental work has been carried out, but the observations of Buddle (1928) at Canton showed the epidemiological relationship of this species to dengue fever. The hospital records at Wuchang indicate that the inhabitants of Wuhan did suffer from this disease, known as " Japanese malaria ", during the Japanese occupation of the area.

Culex (*Lutzia*) *fuscanus* Wiedemann, 1821.

This species is seldom found indoors. Personal observations showed that it probably does not bite humans, and yet Buddle (1928) stated that it bites and sucks human blood greedily, but that its bite leaves no mark. Larvae were frequently found in natural pools together with *Culex tritaeniorhynchus*. Feng (1938) reported that the larvae are predacious, and feed not only on other mosquito larvae, but also on their own kind.

No information regarding its relation to disease in this area is available.

Culex tritaeniorhynchus Giles, 1901.

This species is usually abundant indoors during the latter part of the summer and autumn ; it bites man freely at night, indoors and in the open. Field observations showed that from the end of August onwards breeding was most prevalent in bomb craters, and also took place to some extent in rice fields containing decaying vegetation. Adults were readily obtained in the laboratory from larvae maintained at a temperature of 96·8° to 98·6°F.

Culex vagans Wiedemann, 1828.

Only one specimen was obtained, from the Hankow Union Hospital.

Culex fatigans Wiedemann, 1828.

This is the commonest domestic mosquito in the area, especially during the early part of July. It bites man at night.

Culex pipiens var. *pallens* Coquillett, 1898.

This species attacks humans fiercely at night ; in places where sanitary conditions are poor, it is the one most commonly found in houses. It appears early in November, and remains active even at temperatures at or below freezing point.

Possible transmission of diseases in the area by the five last named species needs investigation.

Ecological Conditions in Larval Habitats.

Samples of water were collected from three distinct breeding sites : a rice field (*Anopheles*) ; a rain-diluted toilet jar (*Armigeres*) ; a pond (*Culex*). In addition, a sample was taken for comparison from a pool where no mosquito larva was ever found.

TABLE II.

Analysis of water from breeding localities of *Anopheles*, *Armigeres* and *Culex*.

	General examination		Chemical analysis				
	Odor	Colour	Residue (mg. /500 ml.)		*pH*	*Total organic nitrogen (mg /l.)	†Dissolved oxygen (mg /l.)
			On evaporation	On ignition			
Anopheles	Grassy, earthy	Muddy yellow	150	120	7·2	0·36	2·23
Armigeres	Urinary	Reddish brown	300	240	8·0	2·88	20·40
Culex	Grassy, earthy	Pale yellow	150	130	7·8	1·20	2·23
Non-breeding	Earthy	Colourless	20	10	7·5	0·96	2·51

* Determined by the Rideal Stewart modification of the Winkler method.
† Determined by the Kjeldahl method.

The samples, each of one gallon, were first filtered, so as to decrease the possibility of further chemical change, before preservation in separate stoppered bottles. Features chosen for analysis were odour, colour, residue, pH value, total organic nitrogen, and dissolved oxygen. The results are shown in Table II.

The significance of these results is not very evident, but the following points may be noted.

As might be expected, the total organic nitrogen in the *Armigeres* breeding water is considerably higher than in the other sites. There may be some connection between this and the rapidity of growth and large size (up to 1 cm. length) attained by the larvae of *Armigeres.*

The amount of dissolved oxygen in the breeding water of *Armigeres* is also very much higher than in the other sites. It has been observed that the larvae of *Armigeres*, in contrast to those of the other species which spend long periods with their syphons open to the air through the surface film, scarcely ever come up to the surface.

In contrast to the breeding water from the other three sites, that in which no breeding took place contained very much smaller amounts of organic substances and minerals.

It seems characteristic of Anopheline larvae that they are most frequently found in association with filamentous algae (*Spirogyra*). Lotus ponds with *Polygonum* along their margins favour the breeding of *Culex* spp. If the water surface is completely covered by floating plants, such as *Azolla, Spirodella, Marsilea, Lemna,* etc., mosquito larvae will not be found.

The fauna of mosquito breeding places may include enemies of the larvae. Tadpoles, fish and aquatic insects are often found as predators on larvae. As mentioned earlier in this paper, larvae of *Culex fuscanus* are recorded as being predacious on other mosquito larvae. During laboratory rearing of larvae of *Armigeres obturbans*, larger larvae were frequently observed to feed on smaller individuals, and also on dead ones.

Larvae of *Anopheles* were generally found in gently flowing water near the passage between two rice fields. Four or five larvae were usually taken in about two square feet of water at such a passage, whilst in still water of equal area scarcely one could be obtained. No *Culex* larvae were found in any moving water.

The larvae of *Anopheles hyrcanus* var. *sinensis* were always found in full sunlight. Larvae of *Culex tritaeniorhynchus* were generally found in the complete shade of water plants and grassy pond edges. Those found in a particular bomb crater were all attached to the adventitious root hairs of *Polygonum* along the shaded bank edge.

The water accumulations in artificial containers which yield larvae of *Aëdes albopictus* are usually poorly illuminated.

Larvae of *Armigeres* develop in almost complete shade, and are highly sensitive to direct sunlight. When exposed to sunlight in the laboratory, all the larvae sank to the bottom of the vessel.

Anopheline larvae thrive in rice fields with a temperature ranging from 70° to 86°F. and tolerate a temperature as high as 104°F. Incubation of the eggs took three days in the laboratory at 83·5°F. When the temperature later dropped to between 66° to 75°F., the larvae stopped growing.

In the laboratory, eggs of *Aëdes albopictus* took two days to hatch, and a total of 12–13 days for the cycle from egg to adult at an average noon temperature of 77·4°F. and at a water temperature of 76·5°F.

Larvae of *Culex tritaeniorhynchus* throve most vigorously at 89·6°F. in a bomb crater in the vicinity of Wuchang. As the temperature gradually dropped, over a period of two weeks, to 66·2°F., it was noted that the larvae became less active and the emerging adults were unable to fly, and some appeared to die.

Larvae of *Armigeres* still survived at temperatures around freezing point, but neither pupation nor emergence of adults occurred.

The figures given in Table I suggest that torrential rain at the peak of the rainy season is less favourable to multiplication of *Anopheles hyrcanus* var. *sinensis* than more moderate intermittent rainfall, with intervals of sunshine, which occur in the latter part of the summer and autumn.

Figures in Table I also suggest that a relative humidity of about 66 per cent. is one of the factors favourable to *Anopheles hyrcanus* var. *sinensis*.

Summary.

Field and laboratory studies were made of the mosquitos of the Wuhan area, central China, between June 1947 and March 1948.

The following species were collected : *Anopheles hyrcanus* var. *sinensis*, *Mansonia uniformis*, *Armigeres obturbans*, *Aëdes albopictus*, *Culex fuscanus*, *Culex vagans*, *Culex fatigans*, *Culex pipiens* and *Culex tritaeniorhynchus*. A brief account is given of the topography and other environmental features of Wuhan.

The seasonal incidence and feeding habits of the adults, and the breeding sites favoured by the various species are recorded.

Monthly mean figures for rainfall, temperature and humidity are examined in relation to incidence of malaria and abundance of adults and larvae of *Anopheles hyrcanus* var. *sinensis*. The peak of malaria incidence coincides with that of *Anopheles* population which occurs two to three months after the peak of rainfall at an average relative humidity of 66 per cent.

A chemical analysis of waters from different types of breeding sites was made. Both the total organic nitrogen, and the amount of dissolved oxygen were considerably greater in the breeding site of *Armigeres obturbans* than in those of other genera. A sample of water from a site in which no mosquito breeding was observed contained very much smaller amounts of organic substances and minerals than samples from known breeding sites.

Other ecological factors affecting the early stages in their breeding sites are briefly referred to.

Acknowledgements.

The writers wish to express their sincere thanks to Dr. C. Ho and Dr. M. K. Hu, both of whom helped in the identification of species, thanks are also due to Dr. L. Weidenhammer, who directed the laboratory analysis of the water samples from breeding sites.

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STUDIES OF THE RESPONSES OF THE FEMALE *AËDES* MOSQUITO.

PART III. THE RESPONSE OF *AËDES AEGYPTI* (L.) TO A WARM BODY AND ITS RADIATION.

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(Plate XVIII.)

Evidence has already been published demonstrating that warmth is a factor in the attractiveness of bodies for adult mosquitos. DeLong and others (unpublished, 1945) reported that the human body surface was attractive to *Aëdes aegypti* (L.) even in an atmosphere of 100 per cent. relative humidity, provided that the air temperature did not exceed 32°C. (89·6°F.): when the air temperature was raised to 34°C. (93·2°F.), the temperature gradient to the skin was so slight that the mosquitos did not respond. It was mentioned by Christophers (1947) that a dry plate warmed to 104°F. proved to be attractive to adult *Aëdes*, and that its attractiveness was only slightly increased further by moistening it. However, Parker (1948) has recently reported that a dry-surfaced conical flask containing water at body temperature failed to attract mosquitos, to the extent of changing their random distribution in a 2-inch tube in which they were confined. But in this case the flask was held 1 cm. away from the screening which closed the end of the tube. Thus only the radiant heat would reach the mosquitos, while the convective heat would be carried up and away without entering the tube.

Many years ago, observations were made by Howlett (1910) to distinguish convective from radiant effects in the attractiveness of warm bodies for various species of Indian mosquitos, including *Aëdes* (*Stegomyia*). He demonstrated that a test-tube filled with warm water, and held obliquely, was attractive to mosquitos only when they were suspended in the convective currents rising from it; there was no response when the mosquitos were held below it or horizontally out from its base. Wigglesworth and Gillett (1934) showed that the attractancy of a test-tube full of warm water to the bug *Rhodnius* was not reduced by wrapping it in aluminium foil despite its eliminating 90 per cent. of the total radiant emissivity.

Recently, Beck and Miles (1947) have adduced evidence to suggest that infra-red radiation is involved in the perception of odours by the cockroach *Periplaneta*. It was claimed that these insects exhibited a response to clove oil vapour even when separated from it by a vapour-tight window of crystalline potassium bromide which was transparent to infra-red radiation in the region of 27 microns wave-length. However, DeLong (unpublished 1949) discovered that the response of *Aëdes* or *Anopheles* adults to arm odour was completely eliminated if they were separated from it by a window of crystalline silver chloride, transparent in the region of 28 microns wave-length.

The present investigation therefore concerned itself initially with establishing the fact that warm bodies are indeed more attractive than cool ones, irrespective of their humidity. The second task was to distinguish between convective and radiant warmth as factors in attractancy. For this purpose a window of thallium bromoiodide, transparent in the region of 25–40 microns wave-length, was interposed between object and insect. In addition, use was made of a Leslie cube containing warm water;

its four vertical faces were prepared with various surfaces of different emissivities, and their respective attractancies to mosquitos were compared.

The insects employed were adult females* of the yellow fever mosquito, *Aëdes aegypti*, which were allowed to fly freely in a large cage of 360 cu. ft. capacity (see Brown, Sarkaria and Thompson, 1951). Early attempts to substitute 4-inch tubes or 12-inch cylinders for the cage were abandoned, since the mosquitos refused to fly unless the glass wall was tapped.

Response to Warm Objects.

Billiard balls were employed as a means of comparing the attractancy of objects of different temperatures. They were warmed for 30 minutes in water at the required temperature ; after being thoroughly dried they were mounted on threaded brass rods, which were centred on a sheet-metal base (Plate XVIII, fig. 1). The two balls to be compared were placed 8 inches apart on a board suspended in the large cage at a height of 4·5 feet from the ground. The room temperature was 75°F. and the relative humidity ranged between 40 and 60 per cent. It was the practice to have the warmer ball at a temperature of 20°F. in excess of the cooler. The numbers of mosquitos which touched each of the two balls over a one-minute period were determined simultaneously by two observers. The balls were alternated between readings, to correct any position error.

The first series of experiments was conducted with a pair of black billiard balls and the second series with a pair of white billiard balls. Each series covered four temperature ranges ; the results are shown in Table I. Each of the figures shown in the table are the totals of ten individual counts (except white, 110–130°F.). The values for the individual counts were employed for statistical treatment to assess the significance of differences. This was performed by analysing the variance and computing the value of Student's t†, from which it is possible to ascertain the probability (p) that such a difference (or more) is due to chance (Mather, 1947).

The data presented in Table I indicate that the attractancy of the billiard balls increased as they were warmed up to 110°F. On the other hand, a ball warmed to 130°F. is less attractive than one at 110°F. The data supporting these conclusions in the case of the black balls are of a high order of significance. It should be stated at this point that 94°F. has been determined to be the skin temperature of a human at rest in air at 75°F.

Response in Absence of Convection.

These experiments were based upon the interposition of an airtight window, transparent to infra-red radiation, between the warm object and the mosquitos. The window employed was a disc of crystalline thallium bromoiodide (also termed a KRS-5 filter) 3 ins. in diameter and 0·5 in. thick, mounted in a bakelite ring. It was placed in airtight fashion over the open end of a metal sleeve, within which a billiard ball could be introduced so that it rested in a central position 2 ins. below the window. The sleeve was 6 ins. in diameter and was mounted obliquely so that the top was 12 ins. within the cage (at a height of 4·5 ft.), but its open lower and projected out of the cage ; thus the ball could be introduced and removed at will by mounting it on the

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* Males were rarely observed in the experiments.

† Derived from the equation :

$$t_{n-1} = \frac{\bar{d}}{\sqrt{\dfrac{\Sigma d^2 - 1/n\ (\Sigma d)^2}{n(n-1)}}}$$

where d is the numerical difference between the two members of a paired count (taking sign into account), and n is the number of paired counts, n–1 representing the degrees of freedom.

TABLE I.

Relative attractancy of warm and cool billiard balls to *Aëdes aegypti.*

Black balls				White balls			
Temp. of each ball °F	Number of mosquitos contacting balls	Index of* attraction (per cent.)	p†	Temp. of each ball °F	Number of mosquitos contacting balls	Index of* attraction (per cent.)	p†
80	209	—		80	281	—	
100	411	66·1	·001	100	408	59·2	·155
90	296	—		90	241	—	
110	1,415	82·7	·001	110	492	67·1	·170
100	542	—		100	460	60·4	·050
120	613	53·1	·650	120	301	—	
110	1,087	61·7	·001	110	71	63·4	·150
130	675	—		130	41	—	

* The percentage of the total count on both balls that contacted the more attractive ball.

† The probability, derived from Student's t value, that this divergence from a 50 per cent. index of attraction was due to chance involved in sampling.

end of a rod (fig. 1). In order to provide a landing-place for the mosquitos, a conical cap of black fine-mesh nylon cloth, 2 ins. high, was placed over the filter.

A black billiard ball warmed to a temperature of 110°F. was inserted in the sleeve, and a count was made of the number of mosquitos which contacted the cone in one

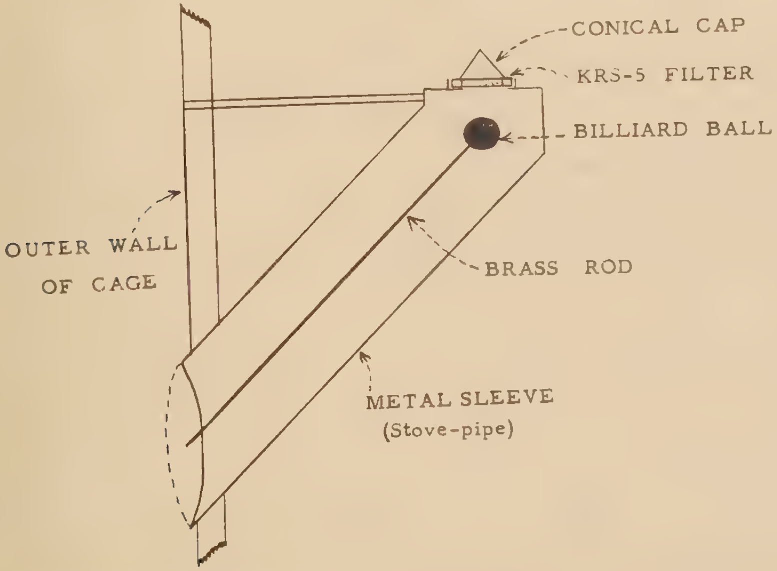

Fig. 1.—Apparatus to test the effect of the interposition of a KRS-5 filter on the response of mosquitos to a warm object.

minute. The ball was then removed, and a control count was made for one minute. Then the ball was returned and the process was repeated several times. The same procedure was followed with the filter removed from its position underneath the cone. Finally the same experiment was repeated, with and without the filter, employing a white billiard ball warmed to 110°F. The results are shown in Table II.

It was noted in this experiment that the female mosquitos remained actively probing through the cone, so long as a filter did not separate it from the ball ; when the filter was interposed or the ball was removed this response disappeared. The results show that the interposition of the window reduces an index of attraction of 80 per cent. for the warm black billiard ball down to a figure of 53 per cent., which is almost a random distribution as if no attractant object were present. The airtight window also reduced the index of attraction of 83 per cent. for the white ball down to 51 per cent., denoting no attractancy.

TABLE II.

The effect of a KRS-5 window on the response of *Aëdes* to the presence and absence of a warm object.

	Black ball				White ball			
	Without filter		With filter		Without filter		With filter	
	Test	Control	Test	Control	Test	Control	Test	Control
Number of mosquitos per 1 min. period	39	10	19	23	3	4	10	12
	41	19	21	8	13	2	9	12
	57	9	8	6	9	4	9	10
	58	7	11	7	26	5	5	5
	50	12	11	17	46	5	8	4
Total	245	57	70	61	97	20	41	43
Index of attraction	81·0%		53·4%		82·9%		51·2%	

Since it appeared that the infra-red window eliminated the attractancy of a single warm billiard ball, it remained to be ascertained whether a pair of windows would abolish the superiority in attractancy, already demonstrated (see Table I), of a warm ball over a cool one. Accordingly a pair of metal sleeves were set up 20 ins. apart each containing a black ball, one at 80°F. and the other at 110°F. It was found (see Table III) that the interposition of these windows reduced the index of attraction of the warmer ball down to 54 per cent., denoting no superiority in attractancy.

TABLE III.

The effect of a KRS-5 window on the preference of *Aëdes* for the warmer of two objects.

	Billiard balls		Water-filled flasks			
	With filter		Without filter		With filter	
	80°F.	110°F.	80°F.	110°F.	80°F.	110°F.
Warm ball on N ...	51	40	29	94	42	40
Warm ball on S ...	44	70	12	110	26	49
Total	95	110	41	204	68	89
Index of Attraction ...	—	54%	—	83%	—	57%

This experiment was repeated with the billiard balls substituted by 50-cc. round-bottom glass flasks covered with black nylon cloth, and filled with water warmed to 80°F. and 110°F. respectively. It was found that the interposition of windows reduced an index of attraction of 83 per cent. for the warmer ball (see Table III) down to a figure of 57 per cent., denoting no significant superiority in attractancy.

Response to Varying Emissivities.

A Leslie cube was employed to provide a source of warmth in which the surface temperature of the four vertical faces would be the same, while the nature of the faces could be varied to result in different emissivities.

Thus the convection rate from each face would be identical whereas the radiation rate would vary according to the nature of the surface. The apparatus is shown in Plate XVIII, fig. 2.

This consisted of a 10-inch cube of polished sheet-metal, filled with water. Its temperature was maintained by means of a knife-type heater and DeKhotinsky thermoregulator, and it was equipped with a thermometer and electric stirrer. The cube rested on the turn-table of a piano stool, which in turn stood on a table in the centre of the cage. The cube could be rotated by the observers from their position outside the cage.

For the series of five experiments which were performed, the metal faces were, either merely polished or else painted with the following materials : flat black paint, flat white paint, black enamel or white enamel ; lamp black from smoking benzene ; clear varnish, or one or two coats of clear lacquer. The attractancy of the painted surfaces was not assessed until they had dried for at least four days. The total radiant emissivities of the resultant faces were measured by a thermopile (Cenco Radiation) and a sensitive galvanometer (Leeds and Northrup, Type R, Model C). The light reflectivity was measured by a photelectric exposure meter (Weston Mode 852) whose maximum sensitivity was to 590 mμ wave-length.

The temperature of the water in the cube was raised to 92°F., and the experiments were performed when the air temperature in the greenhouse compartment was 80°F. and solar radiation was at a minimum. The number of approaches made by the mosquitos to a distance within 1 in. of the face were counted over a one-minute period. The gauging of this distance was assisted by the fact that the 12-in. square platform supporting the 10-in. cube projected 1 in. beyond the face on all sides. Two faces

TABLE IV.

Numbers of mosquitos approaching the faces of a Leslie cube, prepared to offer a range of radiant emissivities.

Expt. No.	Polished metal	Lacquered metal	Lamp black	Black paint	White paint	Black enamel	White enamel
1	645	771					
4	267	291*	257	305			
5	280	201*	169	232			
2	470	498		459	400		
3				1,096	1,038	1,802	1,190
Light reflectivity† ...	2–3	2–3	0·7–1	1	3–8	1·5	8
Heat emissivity† ...	0·15	0·65*	0·7	1	1·05	0·9	1

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* Surface given by a single coat of varnish in Expts. 1 and 2, by a double coat of clear lacquer in Expt. 5 ; in Expt. 4 a single coat of clear lacquer was used, with a relative emissivity of 0·45.

† Measurements of light and heat in empirical, but comparative, units.

were counted simultaneously by two observers ; in experiments 1–3 these faces were adjacent, and in experiments 4 and 5 they were opposite. After each count the cube was rotated into the next position. For any given experiment with the cube as prepared, between 16 and 28 counts were made on each of the four faces. The totals of these counts are shown in Table IV.

From examination of the figures in Table IV it becomes apparent that there is no significant difference between the counts at any of the faces, with the single exception of the black enamel. Where polished metal was compared with lacquered metal, which latter has four times as much emissivity as the former (experiments 1 and 4), there is found to be no significant difference between the counts, analysis of variants revealing that the probabilities of the slight variation observed attributable to chance are 0·300 and 0·400 respectively. The probability that the slight difference in attractancy figure between black paint and the less emissive lamp black was due to chance is 0·600, or 6 out of 10. Most surprising of all, the difference between black and white paint proves to be insignificant ($p<0{\cdot}350$). Evidently when a black surface and a white surface are on the same object there is no difference of attractancy of these areas ; on the other hand, when they were on separate objects, as in the comparison of a white ball with a black ball, the black object proved to be several times as attractive as the white (Brown, Sarkaria and Thompson, 1951).

Black enamel attracted 60 per cent. more mosquitos than white enamel, and the figures indicated that this difference was highly significant statistically ($p<0{\cdot}001$). Moreover, black enamel attracted 70 per cent. more than the flat black paint, and this difference is also highly significant ($p<0{\cdot}001$). It is possible that the reflected images of the mosquitos on the black enamel face may have increased its attractancy.

Summary.

1. A warm object is more attractive to the female yellow-fever mosquito than a cooler object, such that the numbers touching a ball warmed to 100° or 110°F. were twice as great as those touching a ball 20°F. cooler. This attractiveness was reversed when the temperature of the warmer ball reached 120°F.

2. This response is eliminated by the insertion of an air-tight window of thallium bromoiodide, despite the fact that it allows almost all of the radiation to filter through. It is therefore concluded that heat convection, which is eliminated by the air-tight window, is the factor which makes a warm object attractive to the mosquito.

3. The several faces of a warm cube, differing in their radiant emissivity but identical as to surface temperature, exhibited attractancies to *Aëdes* which were not significantly different one from another (except in the case of black enamel, which will be explained in a later paper). These results may be interpreted to indicate that radiation plays no part in the attraction of the mosquitos to the warm face.

4. It is concluded that the failure of Parker (1948) to obtain a positive response of *Aëdes* mosquitos to a warm dry object was due to the fact that his apparatus allowed only the radiant heat but not the convective heat to reach the insects.

5 Since the response of *Aëdes aegypti* to heat is eliminated by a window transparent to infra-red radiation, the theory of olfaction proposed by Beck and Miles (1947) evidently has no application to this species of mosquito.

Acknowledgements.

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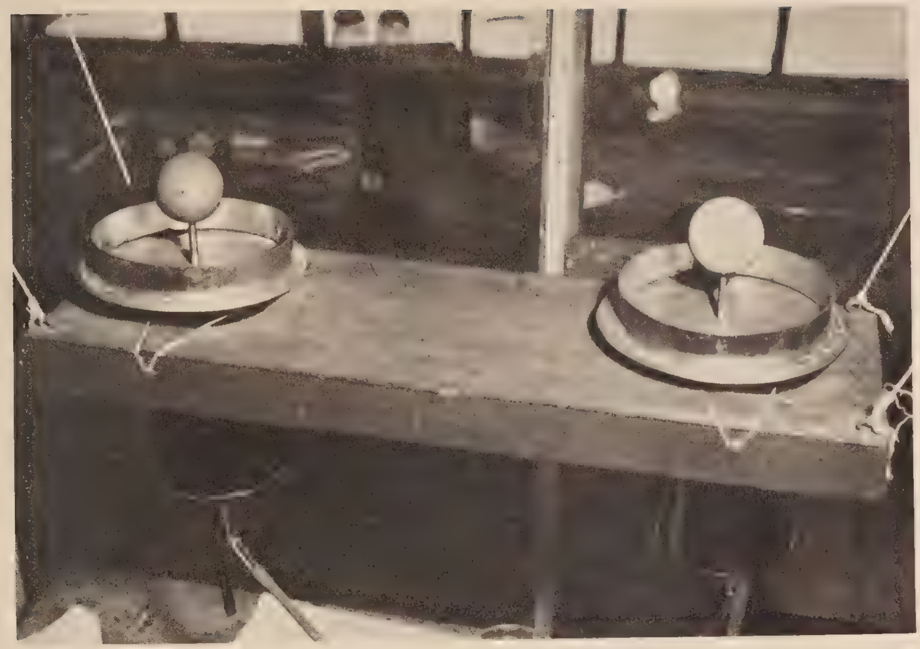

FIG. 1. Billiard balls mounted on a board in a large cage, to assess the response of mosquitos to a warm object.

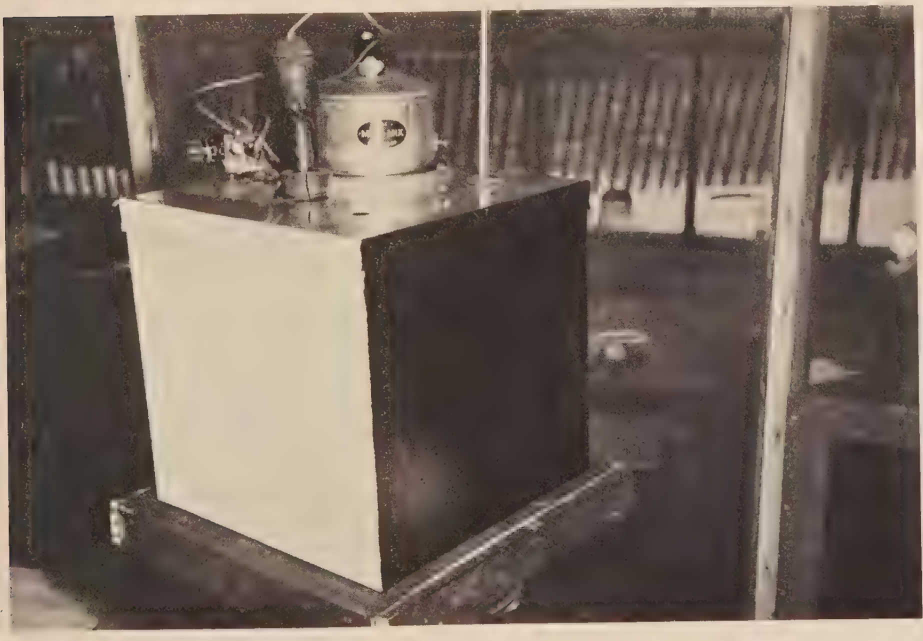

FIG. 2. A Leslie cube, used to assess the response of mosquitos to faces of identical temperature but various radiant emissivities.

CONTROL OF HUMIDITY WITH POTASSIUM HYDROXIDE, SULPHURIC ACID, OR OTHER SOLUTIONS.

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Since the publication of the well-known papers by Buxton (1931) and Buxton & Mellanby (1934), the control of atmospheric humidity in biological experiments by means of sulphuric acid or potassium hydroxide solutions has been increasingly widely practised. The frequency of references to these papers testifies to the important part they have played in making the methods available to biologists.

The chief purpose of the present paper is to provide convenient access to up-to-date and accurate data on solutions useful for humidity control. Reference is made to some of the pitfalls associated with their use, and convenient methods of preparing solutions are indicated.

To avoid confusion, it may be necessary to give the accepted definition of *relative humidity* as the ratio of the actual vapour pressure to the saturation vapour pressure at the same temperature, expressed as a percentage. The *water activity* (sometimes called *relative vapour pressure*) is the same ratio, expressed as a decimal of unity. The relative humidity is only approximately equal to the *percentage saturation*, or weight of moisture in the air as a percentage of that in saturated air at the same temperature.

In dealing with the concentrations of solutions, measures referred to will include *weight per cent.* (wt.%), which is g. of pure solute in 100 g. of solution, and *molality*, which is the number of gram-molecules of solute per 1,000 g. of solvent ; molality is to be distinguished from *molarity*, which is gram-molecules per litre of solution.

The terms *specific gravity* and *density* are both used in this paper. For a given temperature, their values are identical if the density of water at 4°C. is taken as the basis of specific gravity.

To save space, the following contractions are used for three sources frequently referred to :—

I.C.T. for International Critical Tables,
B.S.I. for publications of the British Standards Institution,
H.C.P. for the Handbook of Chemistry and Physics.

Choice of Method, and Some Precautions.

There are two alternative ways of providing a series of humidities by the use of solutions. The first is to make up, from a suitable compound, a series of solutions at concentrations adjusted to provide the appropriate water vapour pressures. The second is to use saturated solutions of different salts which can be selected according to the humidities required.

Only the first method will give a precise and evenly graded series, such as a set of humidities at 5 per cent. intervals. If low humidities are to be included in the range, one must use some strongly hygroscopic material such as potassium hydroxide, sodium hydroxide, or sulphuric acid (these give humidities down to about 13 per cent., 5 per cent. and zero respectively, at normal working temperatures). Such substances evolve a great deal of heat when dissolved or diluted in water. The acid is the most dangerous if the proper precautions are neglected, *e.g.*, if water is poured into a concentrated solution, instead of *vice versa* ; but all behave violently if misused, and require heat-resistant glassware for the earlier stages of dilution. All are dangerously corrosive and burn the skin if not washed off promptly.

The physical data for sulphuric acid solutions are probably more accurately determined than the data for other compounds. But the alkalis have the property, attractive to biologists who keep experimental insects, etc., under controlled humidity conditions in desiccators, of absorbing carbon dioxide. This reduces the frequency with which the desiccators have to be opened for aeration, but they must still be opened often enough to prevent lack of oxygen ; also, in time, the absorption of carbon dioxide with the formation of carbonate and water leads to a change in the equilibrium humidity over the solution.

With any sort of graded humidity solutions, errors arise if they lose too much water through absorption of water vapour by materials enclosed with the solutions, or if they absorb much water vapour through being enclosed with damp materials.

A common source of inaccuracy is variation in the ambient temperature. During the diurnal variations of temperature in an uncontrolled room, the temperature of the solution changes more slowly than that of the air space above it. The consequent temperature differences cause deviations from the expected humidity. Hence accurate humidity control with graded solutions is possible only at constant temperature. A temporary change in humidity will inevitably occur whenever the desiccator is removed to another temperature, *e.g.*, for examination.

Similar considerations apply to a saturated salt solution : its equilibrium humidity deviates from the expected value if the solution is at a different temperature from that of the air above it.

In general, solutions will tend to give too low a humidity when the ambient temperature is rising, and *vice versa.*

Even although it is usual to keep saturated salt solutions in contact with an excess of the solid salt, in still conditions the solutions may become unsaturated at the surface by absorption of water vapour from materials enclosed with them, and so give too high a humidity. Naturally they can no longer prevent a fall in humidity if allowed to become dry, or covered over with a layer of crystals.

After desiccators, etc., have been opened, the humidity inside may take some time after replacement of the lid to return to the equilibrium level, particularly if the internal arrangement creates " dead spaces ". Cobalt thiocyanate papers are useful as indicators of the humidity inside specimen tubes, etc., when errors of this sort are expected (Solomon, 1945). There is no doubt that the installation of a small internal fan would often be an improvement. Beattie (1928) used a rubber bulb, operated by hand. An alternative is to arrange a flow of conditioned air through the apparatus (Solomon, 1937 ; Johnson, 1940 ; Schoof, 1941).

Preparation of Graded Solutions.

By hydrometry.

A common method of preparing graded solutions for humidity control is by making them up to the required densities, using hydrometers.

With substances such as potassium hydroxide or sodium hydroxide, the first step is to dissolve the solid in distilled water. For accurate control, there may be some advantage in using material of the greatest available purity, and in using sticks instead of pellets, since the latter have a larger surface area and probably contain a correspondingly greater amount of carbonate. Heat resistant glassware should be used, as also for the dilution of concentrated sulphuric acid.

Since the density of a solution changes with temperature, the temperature at which the density is measured must be taken into account. For example, if we require a sulphuric acid solution of density 1·20 at 20°C., either the density must be measured at that temperature, or else we must consult tables or graphs to find what the density of such a solution is at the temperature of measurement.

A further small temperature correction may be necessary if the hydrometer is used at a temperature other than that for which it has been calibrated. At 10°C. above the calibration temperature, a density hydrometer may read below the true value by as much as 0·0005 g./ml., with a reverse error of the same order at 10° below the calibration temperature.

Variations in surface tension may also lead to errors if corrections are not applied. On this point, as on all aspects of the use of density hydrometers, B.S.I. No. 718 should be consulted. The table on p. 31 of that booklet shows corrections up to 0·0035 g./ml. for differences in surface tension. The use of an overflow vessel is recommended, to ensure a clean surface on the solution during hydrometry.

Sets of hydrometers of widely different sensitivities are available. One in which each hydrometer covers a range of 0·05 g./ml. gives results sufficiently accurate for most purposes. Sets of British Standard Hydrometers are also made. These differ in sensitivity, but all are calibrated for 20°C. In the B.S.I. booklets of density-composition tables for solutions of sulphuric acid, caustic soda, sodium chloride, and calcium chloride, respectively, there are precise directions for the use of hydrometers with these solutions, but there is no such booklet devoted to potassium hydroxide solutions.

If the hydrometer shows that the solution is not at the density required, more water or more concentrated solution is added. The solution must then be stirred, cooled again if necessary to a suitable temperature, and re-stirred, before another hydrometer reading is made. After each immersion, the hydrometer should be thoroughly washed in water, and dried. The above process is repeated until the correct density is reached.

Buxton & Mellanby (1934) remarked that an alternative to hydrometry is to weigh a known volume of the solution, discharged from a pipette. If the draining procedure is standardised, this method may give results accurate enough for many purposes; but drainage errors would be considerable with strong solutions. This might be avoided by weighing liquid and pipette together, or by using a small standard flask. In any case, it is best to weigh also a volume of distilled water, measured out in the same way, and to convert the results from specific gravity to density; in this way buoyancy errors, due to weighing in air, are also reduced.*

It may be worth noting that, over most of the humidity range, an error of 0·001 in the density of a solution makes a humidity difference of about 0·2 per cent. R.H. with potassium hydroxide, and 0·1 per cent. R.H. with sulphuric acid.

A gravimetric method.

In preference to trial and error methods of approaching the desired densities, and to avoid the repeated use of hydrometers and repeated stirring and cooling of the solutions, I have used a gravimetric method involving a simple calculation.

The first step is to make up a large stock solution, stronger than any of those required for humidity control. This is brought to a convenient temperature, stirred, and its density determined as accurately as possible by several successive readings

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*In tables of densities and wt. per cent., it is usually the weight *in vacuo* which is given. For highly accurate work, weights should be determined *in vacuo* or else buoyancy corrections should be made. Without this precaution, density determined by weighing would not be the same as specific gravity; to arrive at the real weight of a solution, one must add about 0·1 per cent. to the weight in air (cf. B.S.I. No. 718, p. 48).

of the hydrometer. To make up a solution of any specified density, some of this stock solution is poured into a vessel, and its weight determined on a balance. Then the quantity of water needed to bring the aliquot of stock solution to the required density is calculated, the correct amount weighed out, and the two lots of liquid mixed together. (Alternatively, the water could be measured out, probably less accurately, from a burette.)

The calculation of the required quantity of water depends on the fact that the weight of water w to be added to each gram of a stock solution of wt.% S, to bring it to a reduced wt.% A, is given by the relationship $w=\frac{S}{A}-1$. S and A are read off from tables connecting wt.% and density, or from a graph of such data (*cf.* Table II and fig. 1). The value of w, when multiplied by the weight of stock solution in the aliquot, gives the quantity of water to be weighed out and added.

Volumetric methods.

For sulphuric acid solutions, a somewhat similar procedure was described by Buxton & Mellanby (1934). Taking the specific gravity of analytical grade concentrated acid as 1·841, they mixed together equal volumes of concentrated acid and water, to deal with most of the heat evolution in one operation. Then they added measured volumes of this stock solution to measured volumes of water, and showed in a table the specific gravities of the resulting solutions. This method is undoubtedly convenient, and should be accurate enough for most purposes provided the original concentrated acid, and the stock solution, are really of the specific gravity expected. It should be noted, however, that according to the more modern data used in the present paper, there are considerable inaccuracies in Buxton & Mellanby's table, both as regards the specific gravities got by mixing in the proportions stated, and the humidities corresponding to the specific gravities given.

Anyone wishing to use this method can readily prepare a table of data, provided the density and wt.% of the stock solution are known. For approximate work, the wt.% of the stock solution may be calculated from the specific gravity and wt.% of the concentrated acid, as given on the label. For example, if these are given as 1·84 and 98 per cent., the wt.% of a stock solution made by adding acid to an equal volume of water should be

$$\frac{1{\cdot}84\times 98}{1{\cdot}84+1}=63{\cdot}49$$

The density corresponding to this wt.% may be read from a graph, like fig. 1, made from the data in Table III ; the value is 1·540.

To calculate the proportions by volume of stock solution and water required for a solution to give a particular humidity, say 40 per cent. R.H., we must read off from the graph, or from Table III, the wt.% corresponding to this R.H. ; it is 47·71. Then the required volume ratio is 1 part of stock solution to v parts distilled water, where

$$v=d\left(\frac{S}{A}-1\right),$$

d being the density of the stock solution, S its wt.%, and A the wt.% of the solution required. In the present example,

$$v=1{\cdot}54\left(\frac{63{\cdot}49}{47{\cdot}71}\right)-1=0{\cdot}510$$

The proportion of stock solution and water required to provide other relative humidities can be calculated in the same way. For more accurate work, it would, of course, be necessary to measure the density of the stock solution directly.

This volumetric method may be followed in the same way with potassium hydroxide or any other material, given a stock solution of which the density has been determined, and a set of data like those in Tables II and III.

There are tables which give the proportions of sulphuric acid and water, by volume, required to make solutions of particular specific gravities. I have examined only one of these (in H.C.P.) and found it too inaccurate for humidity solutions.

Data for Solutions of Potassium Hydroxide.

The vapour pressures over potassium hydroxide solutions are given in I.C.T. **3**, 373, for temperatures 0°, 20°, 40°C. and higher ; 13 papers are cited as the source of these figures. The data for the four lowest temperatures are given in Table I.

Similar figures for 0° and 25°C. may be calculated from data given in I.C.T. **3**, 298 and for 25° from data in Landolt-Börnstein, Aufl. 5, Ergbd. 3, Teil 3, 2524. The two sets of data for 25° are each based on a single author.

In Table I, since the top figure of each vapour pressure column represents the saturation vapour pressure over water, the relative humidities are calculated by expressing each of the lower figures as a percentage of this.

The relationship between concentration (wt.%) and density at 15°C. is given by another set of data in I.C.T. **3**, 86. This is based primarily on the work of Pickering (1894), but 20 other references are cited. Pickering's data are also given in Landolt-Börnstein, Aufl. 5, Bd. 1, 400, and a table based on the I.C.T. is given in H.C.P.

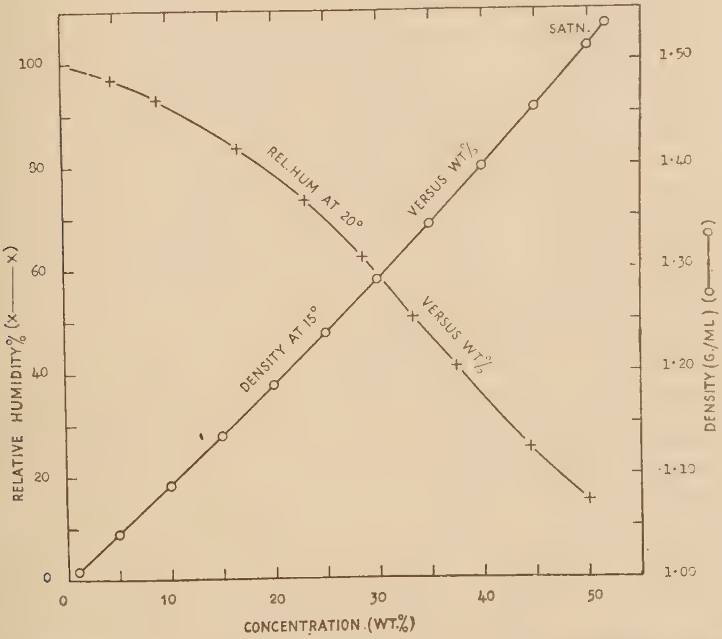

Fig. 1.—Graph of data from Table I, for relative humidity over solutions of potassium hydroxide at 20°C. *versus* concentration of solution shown thus —+——+.

Graph of data from I.C.T. **3**, 86, for density at 15°C. *versus* concentration of solution shown thus—o——o.

Readings may be made up or down (but not sideways) from one curve to the other.

We thus have available a set of figures relating relative humidity, through vapour pressure, to concentration, and another set relating concentration to density. The two sets are graphed in fig. 1. In this diagram the scale on the right refers only to the density curve, that on the left only to the humidity curve, while the horizontal scale refers to both. To illustrate the way of using this type of diagram, suppose we require to know the humidity given by a potassium hydroxide solution of unknown concentration. Its density is measured with a hydrometer and found to be (say) 1.239. Reading across from this point on the scale to the density curve, and thence down to the wt.% scale, we find this density corresponds to wt.% 25·0. Reading upwards from this point to the humidity curve, and thence across to the left-hand scale, we find the corresponding relative humidity, which is 70 per cent. In a case such as this, we need not have referred to the wt.% scale at all, but could have read upwards from the density curve to the humidity curve, and thence across to the left-hand scale ; readings may be made vertically upwards or downwards (but not sideways) from either curve to the other.

In fig. 1 the density curve is the one for 15°C. The humidity curve, on the other hand, gives the relative humidity at 20°C. ; if the curves for 0° and 40° are also drawn in, the humidities given at temperatures above or below 20° may be estimated by interpolation, or such corrections may be estimated from Table I.

Whether for general reference or for the drawing of a graph such as fig. 1, the data given in Table II will be found convenient. They have been read off from such a graph, constructed from the above-mentioned data in I.C.T.

Data for Solutions of Sulphuric Acid.

There is a fairly extensive literature containing data for vapour pressures over sulphuric acid solutions. One gathers from this that, until very recent years, no accurate set of data has existed. But modern work with more adequate techniques has shown that precision can be achieved. Shankman & Gordon (1939) produced data for relative humidities at 25°C., which are adequate for the drawing of a curve. The new data of Stokes & Robinson (1949) agree very well with these, and cover almost the same range of humidity (5 per cent.–95 per cent.) ; they are given in the first two columns of Table III. The series can be extended upwards if some of Shankman & Gordon's figures in their Table II are converted from molality (see p. 1) to wt.%. For humidities below 5 per cent., the vapour pressure tables in I.C.T. **3**, 303 may be used. For the greater part of the humidity range, the I.C.T. vapour pressure figures agree fairly closely with the above newer data, but give humidity values above 80 per cent. which are too low, at some points by more than 1 per cent. R.H.

Vapour pressure data for other temperatures below 50°C. are given in I.C.T., but the relations between temperature and humidity over any given solution, when calculated from these tables, are most erratic. Probably the data of Collins (1933) are more reliable ; although they were criticised by Shankman & Gordon (*l.c.*), they at least appear self-consistent. Table IV gives a set of corrections to be applied to humidity values when the solutions are not at 25°C., based on Collins' figures. He did not deal with temperatures below 20°C.

A table of densities of sulphuric acid solutions was given by Shelford (1929, p. 252), quoting figures used by Stevens (1916). These agree well with the values given in I.C.T. **3**, 56–7 for 15°C. The I.C.T. tables cover a series of temperatures, as do those in B.S.I. No. 753, which are based upon them ; these sources provide the data for curves of concentration *versus* density at 15° and 20°C. The third and fourth columns of Table III comprise readings from such graphs. The Table as a whole gives the figures necessary for the making of a diagram like fig. 1, or for the preparation of solutions to give the humidities in the first column.

TABLE I.

Data from I.C.T. **3**, 373, showing relationship between concentration and vapour pressure of potassium hydroxide solutions.

Concentration of solution.		Water vapour pressure over solutions, in mm. Hg., and equilibrium relative humidities.							
		At 0°C.		At 20°C.		At 40°C.		At 60°C.	
g. KOH per 100 g. water in solution	Wt. % of solution, *i.e.*, g. KOH per 100 g. solution	V.P.	R.H. per cent.	V.P.	R.H. per cent.	V.P.	R.H. per cent.	V.P.	R.H. per cent.
0	*0*	4·579	*100*	17·539	*100*	55·34	*100*	149·46	*100*
5	*4·76*	4·43	*96·75*	17·0	*96·93*	53·5	*96·68*	144·6	*96·75*
10	*9·09*	4·25	*92·81*	16·3	*92·94*	51·4	*92·88*	138·9	*92·93*
20	*16·67*	3·83	*83·64*	14·7	*83·81*	46·4	*83·85*	125·4	*83·90*
30	*23·08*	3·35	*73·14*	12·9	*73·55*	40·7	*73·55*	110·1	*73·67*
40	*28·57*	2·82	*61·58*	10·9	*62·15*	34·6	*62·52*	94·0	*62·89*
50	*33·33*	2·30	*50·23*	8·9	*50·75*	28·6	*51·68*	78·6	*52·59*
60	*37·50*	1·82	*39·75*	7·2	*41·05*	23·2	*41·92*	64·6	*43·22*
80	*44·44*	1·08	*23·58*	4·4	*25·09*	14·6	*26·38*	42·6	*28·50*
100	*50·00*			2·6	*14·82*	8·8	*15·90*	26·5	*17·73*
120	*54·55*					5·2	*9·40*	16·2	*10·84*
140	*58·33*							9·8	*6·56*

The columns showing wt % of solution and relative humidity (figures in italics) have been calculated from these data by the author, to the nearest 0·01.

Other Graded Solutions.

An alternative to potassium hydroxide is sodium hydroxide. Other compounds which have been used in the same way for humidity control are sodium chloride, calcium chloride, and glycerol. The following notes give sources of data on the basis of which suitable solutions may be prepared.

For calcium chloride and sodium hydroxide solutions, the relationships between wt.% and the density at a range of temperatures is given in tables in I.C.T. **3,** 72–3 and 79. The similar tables in B.S.I. Nos. 823 and 824 are derived from these, and so are the tables in H.C.P. which cover only one temperature, 20°C. The relationships between wt.% and water vapour pressure over a range of temperatures are set out in I.C.T. **3,** 368 and 369–70. Nomographs connecting molality and vapour pressure over sodium hydroxide at temperatures 0°–70°C. are given by Davis (1942), using recent data. Stokes & Robinson (1949) give a useful table relating wt.%, molality and relative humidity, for both compounds, but for only one temperature, 25°C.

TABLE II.

Potassium hydroxide solutions for control of atmospheric humidity.

R.H. % at 20°C.	Wt. % (g. KOH per 100g. soln.)	Density (g. /ml.) at 15°C.
100	0	1·00
95	7·00	1·064$_0$
90	11·75	1·108$_5$
85	15·80	1·147$_5$
80	19·25	1·181$_0$
75	22·25	1·211$_0$
70	25·00	1·239$_0$
65	27·30	1·262$_5$
60	29·50	1·285$_0$
55	31·60	1·307$_5$
50	33·70	1·330$_0$
45	35·80	1·353$_0$
40	37·95	1·378$_5$
35	40·10	1·400$_0$
30	42·30	1·425$_0$
25	44·50	1·450$_0$
20	47·00	1·479$_0$
15	49·80	1·512$_0$

Concentration (wt. % figures read from graph of 20°C. data in Table I. Density figures read from graph of 15°C. data in I.C.T. **3,** 86. Both graphs shown in fig. 1.

For humidities above 75 per cent., sodium chloride solutions may be used. The relationship between wt.% and density over a range of temperatures is given in I.C.T. **3,** 79. The similar information in B.S.I. No. 823 is based on this, as is the table in H.C.P. which covers only 20°C. The relationship between wt.% and water vapour pressure over a range of temperatures is given in I.C.T. **3,** 370. (Critical studies of the vapour pressure data for sodium chloride have been made by Harned & Cook (1939) and Janis & Ferguson (1939).)

Johnson (1940) used glycerol solutions for humidity control. The data, taken from Landolt-Börnstein, are set out in his paper. Tables for the densities of glycerol solutions at a range of temperatures are given in I.C.T. **3,** 121–2, and vapour pressure data in I.C.T. **3,** 291, 293. Some of these vapour pressure data, however, were criticised by Grover & Nicol (1940), who gave tables and a graph relating wt.%, vapour pressure, relative vapour pressure, and the refractive index of solutions.

TABLE III.

Sulphuric acid solutions for control of atmospheric humidity.

R.H. % at 25°C.	Wt. % (g. H_2SO_4 per 100 g. solution.)	Density (g. /ml.) at 15°C.	Density (g. /ml.) at 20°C.
100	0	$0{\cdot}999_1$	$0{\cdot}998_2$
95	11·02	$1{\cdot}075_5$	$1{\cdot}073_5$
90	17·91	$1{\cdot}126_0$	$1{\cdot}123_0$
85	22·88	$1{\cdot}165_0$	$1{\cdot}162_0$
80	26·79	$1{\cdot}196_5$	$1{\cdot}193_5$
75	30·14	$1{\cdot}223_0$	$1{\cdot}219_5$
70	33·09	$1{\cdot}248_5$	$1{\cdot}245_0$
65	35·80	$1{\cdot}271_0$	$1{\cdot}267_5$
60	38·35	$1{\cdot}292_5$	$1{\cdot}289_0$
55	40·75	$1{\cdot}313_0$	$1{\cdot}309_5$
50	43·10	$1{\cdot}334_0$	$1{\cdot}330_0$
45	45·41	$1{\cdot}355_5$	$1{\cdot}351_5$
40	47·71	$1{\cdot}377_5$	$1{\cdot}373_5$
35	50·04	$1{\cdot}400_0$	$1{\cdot}396_0$
30	52·45	$1{\cdot}423_5$	$1{\cdot}419_5$
25	55·01	$1{\cdot}449_5$	$1{\cdot}445_5$
20	57·76	$1{\cdot}478_0$	$1{\cdot}474_0$
15	60·80	$1{\cdot}511_0$	$1{\cdot}507_0$
10	64·45	$1{\cdot}551_0$	$1{\cdot}546_5$
5	69·44	$1{\cdot}608_5$	$1{\cdot}604_0$

The figures in the first two columns are from Stokes & Robinson (1949). The third and fourth columns comprise readings from the curves of concentration *versus* density at 15° and 20°C., based on the data in I.C.T. **3**, 56–7.

Saturated Salt Solutions.

The humidity of air in contact with saturated solutions of various salts are given in Landolt-Börnstein, I.C.T., H.P.C., and elsewhere. Some of those suitable for biological use were listed by Buxton & Mellanby (1934).

Most of this information has been brought together and conveniently tabulated by O'Brien (1948), who lists the humidities given by various salts at different temperatures, with remarks on their suitability for biological and general use.

Stokes & Robinson (1949) give some new data for the equilibrium relative humidity in contact with saturated solutions of certain salts at 25°C., as follows :—

$K_2Cr_2O_7$	98·00 per cent.	$NaBr.2H_2O$	57·7 per cent.
KNO_3	92·48 „ „	$Mg(NO_3)_2.6H_2O$ ...	52·86 „ „
$BaCl_2.2H_2O$	90·19 „ „	$LiNO_3.3H_2O$	47·06 „ „
KCl	84·26 „ „	$K_2CO_3.2H_2O$	42·76 „ „
KBr	80·71 „ „	$MgCl_2.6H_2O$	33·00 „ „
NaCl	75·28 „ „	$K(C_2H_3O_2).\,1{\cdot}5H_2O$ ...	22·45 „ „
$NaNO_3$	73·79 „ „	$LiCl.H_2O$	11·05 „ „
$SrCl_2.6H_2O$	70·83 „ „	$NaOH.H_2O$	7·03 „ „

Carr & Harris (1949) also give new data for saturated solutions over a range of temperatures. The following are the relative humidities given for the lowest temperature in each of their tables :—

KCl	85·0 per cent.	at 20·2°C.
KBr	79·6 „ „	„ 40·0°C.
NaCl	75·9 „ „	„ 15·5°C.
$NaNO_3$	72·8 „ „	„ 30·0°C.
KI	66·8 „ „	„ 40·0°C.
$Na_2CrO_4.4H_2O$ ...	64·6 „ „	„ 30·2°C.
$NaNO_2$	63·0 „ „	„ 30·1°C.
$NaBr.2H_2O$	58·0 „ „	„ 20·3°C.
$Na_2Cr_2O_7.2H_2O$ ...	54·2 „ „	„ 30·0°C.
CrO_3	45·4 „ „	„ 15·0°C.
$NaI.2H_2O$	36·4 „ „	„ 30·2°C.
$KF.2H_2O$	27·4 „ „	„ 30·4°C.

TABLE IV.

Corrections to be applied to the relative humidity values expected over solutions of sulphuric acid, when the temperature is not 25°C. ; based on data of Collins (1933).

Wt. % of solution :—		10	20	25	30	35	40	45	50	55	60	65	70
Approximate R.H. at 25°C. :—		96%	88%	82%	75%	67%	57%	46%	35%	25%	16%	9%	4%
R.H. decrements or increments at—	20°	0	0	−0·1	−0·2	−0·3	−0·4	−0·5	−0·5	−0·5	−0·5	−0·5	−0·3
	30°	0	0	+0·1	+0·2	+0·3	+0·4	+0·5	+0·5	+0·5	+0·5	+0·4	+0·4
	35°	0	+0·1	+0·3	+0·4	+0·6	+0·8	+1·0	+1·1	+1·0	+0·9	+0·8	+0·7
	40°	0	+0·1	+0·4	+0·6	+1·0	+1·2	+1·4	+1·6	+1·6	+1·4	+1·3	+1·1
	50°	0	+0·2	+0·6	+1·0	+1·6	+2·0	+2·4	+2·6	+2·7	+2·4	+2·1	+1·7
	60°	0	+0·3	+0·8	+1·4	+2·2	+2·8	+2·8	+3·7	+3·8	+3·4	+3·0	+2·5

Summary.

Methods of preparing solutions of graded density for the accurate control of atmospheric relative humidity are described, and some pitfalls in their use and in the use of saturated salt solutions are indicated.

For graded solutions of potassium hydroxide and of sulphuric acid, data from the International Critical Tables or more recent sources are used as the basis of tables giving the concentrations (wt.%) and densities corresponding to relative humidities in steps of 5 per cent. R.H. Sources of similar data for calcium chloride, sodium hydroxide, sodium chloride, and glycerol solutions are given.

As an addition to the compilation of the available data on humidities in contact with various saturated salt solutions by O'Brien (1948), some more recent figures are quoted.

Acknowledgement.

The material of this paper is partly a compilation from the literature, made in connection with the work of the Pest Infestation Laboratory, together with notes and suggestions from experience gained chiefly in the course of this work, and is published by permission of the Department of Scientific & Industrial Research.

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THE SEPARATION OF *CONWENTZIA PINETICOLA* END. FROM *CONWENTZIA PSOCIFORMIS* (CURT.), AND NOTES ON THEIR BIOLOGY.*

By Elsie Collyer.
East Malling Research Station.

(Plate XIX.)

A study of the predators of the Fruit Tree Red Spider Mite, *Metatetranychus ulmi* (Koch), in the orchards of Essex has been in progress since 1946. In 1947 and subsequently, a species of the family Coniopterygidae (Neuroptera) was found, often plentifully, preying on the mites. Massee (1946) had previously reported cocoons of *Coniopteryx tineiformis* Curt. in Essex orchards, but examination of the larvae found in 1947 showed that they belonged to a distinct species, which proved on further study to be *Conwentzia pineticola* End., the early stages of which have hitherto been very inadequately known, and which, on the characters of the adults only, has been regarded by one author as merely a variety of *Conwentzia psociformis* (Curt.). The present writer wishes to point out that in a previous paper (Collyer, 1949) the species called *Conwentzia psociformis* (Curt.) was actually *Conwentzia pineticola* End. as described in the present paper.

The larvae that were abundant on apple trees in 1947, in August and September, and which were later found in Kent as well as Essex, were superficially like those described by Killington (1936) and other workers for the genus *Coniopteryx*. The adults which developed from these larvae, however, had the reduced pair of hind wings characteristic of the genus *Conwentzia*, and could not be confused with *Coniopteryx*, all species of which have two pairs of subequal wings. Withycombe (1923) drew attention to a structural character for separating the larvae of *Conwentzia* and *Coniopteryx* ; the pair of setae which project over the tarsal claws are fine and tapering in *Conwentzia*, but end bluntly in *Coniopteryx*. The larva discussed here has fine and tapering setae, and therefore, despite its general appearance, belongs to the genus *Conwentzia*. According to Killington, only one species of this genus is represented in the British Isles. This is *Conwentzia psociformis* (Curt.), of which he describes a form *pineticola* End. ; Enderlein (1905, 1907) described *Conwentzia pineticola* as a separate species on the basis of the adult characters alone. Withycombe (1922, 1923) treats *pineticola* as a separate species in two papers, but expresses doubt as to whether it is actually a species or merely a biological race ; he considered both larval and adult characters, and found the latter of more importance in distinguishing the two forms.

Separation of the Adults.

The true *C. psociformis* was found and studied in two localities in the present investigation. Both the adult and the larva were found to agree with Killington's description. The adults of *psociformis* and *pineticola* were compared and the following four characters considered ; the first two of these had been used by Enderlein (1905) for separating the two species. The number of specimens studied was 20 in the case *psociformis* and 40 in that of *pineticola*.

Number of antennal joints.

Table I shows that this is a variable character, and although two groups of adults can be separated on it, individual specimens with 36 joints are indeterminable.

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*Part of a Thesis approved for the Degree of Doctor of Philosophy in the University of London.

TABLE I.
Number of antennal joints.

	Maximum	Minimum	Average
C. psociformis	43	36	38
C. pineticola	36	28	32
C. psociformis } (after Enderlein)	43	38	
C. pineticola } (after Enderlein)	34	28	32–33

Venation.

Comparison of the venation again separates the adults into two main groups, although many intermediate forms occur ; the chief variations are shown in fig. 1. Thirteen of the specimens of *C. psociformis* examined show the cross-vein between R_1 and Rs in the fore-wing joining the upper arm R_{2+3} of the forked Rs. (fig. 1A), and this is the typical venation for this species as described by Enderlein ; in the remaining seven specimens, however, the cross-vein strikes Rs at the fork-point (B). In the case of *C. pineticola*, 17 of the specimens show the cross-vein joining the stem Rs before the fork-point (C), while in 20, the point of junction is at the fork-point (D) ; in the remaining three it is beyond it (E). In the group of specimens represented by fig. 1 D, the cross-vein from Sc to R_1 is considerably beyond that from R_1 to Rs in most specimens, but in four of them it is almost in line with it, and this latter variation corresponds to *Conwentzia cryptoneuris*, which was described as a new species by Bagnall (1915). Arrow (1917) states that there is no reason for separating *Conwentzia cryptoneuris* Bagnall from *Conwentzia psociformis* (Curt.), and Withycombe (1922) regards it as synonymous with *Conwentzia pineticola* End. Variations in the position of other cross-veins have been noted, but they are not considered here as they are even less reliable than those described.

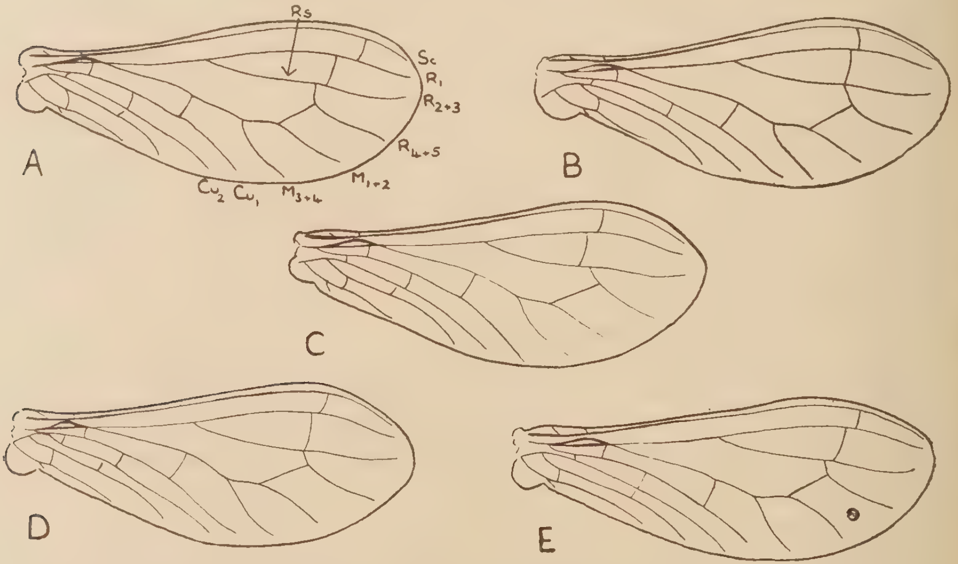

Fig. 1.—Venation of fore-wing. A and B : *Conwentzia psociformis* (Curt.). C, D. and E. : *Conwentzia pineticola* End.

Male genitalia.

The structure of these organs has been considered a good specific character in the Coniopterygidae, and the male genitalia of *C. psociformis* have been figured by Withycombe (1922) and Killington (1936). In the present investigation, the male genitalia of *C. pineticola* were found to be identical with those of *C. psociformis*.

Size.

The adults vary considerably in size, but there is little difference between the averages for the two species.

	C. psociformis	*C. pineticola*
Wing expanse ...	7·0–8·3 mm.	6·2–8·8 mm.
Average	7·9 mm.	7·6 mm.

On the basis of these four characters, the two forms found in Essex are respectively very similar to *C. psociformis* and *C. pineticola* as described by Enderlein (1905, 1907) and Withycombe (1922, 1923). If large numbers of both forms are available, they can be separated on the number of antennal joints and on venation, but there are many intermediate forms which cannot be separated individually on either of these characters with certainty. These two characters are recognised as being very variable throughout the Coniopterygidae, and in fact the two fore-wings of a single specimen frequently differ in venation. It is hardly suprising, therefore, that Killington placed *pineticola* as merely a form of *C. psociformis*.

Separation of the Larvae.

Withycombe (1923) compared the larvae of *C. psociformis* and *C. pineticola*. He states that the early stages of the two are indistinguishable, except that the antennae of *C. pineticola* are somewhat shorter, averaging 0·40 mm. in length as compared with 0·75 mm. He finds, however, no reliable distinction, and states that larvae of the *psociformis* type give rise to adults which produce larvae of the *pineticola* type. He regards them as occurring in separate localities, but states that they do not breed true. In a paper on the Fruit Tree Red Spider Mite in Finland by Listo, Listo and Kanervo (1939), *Conwentzia pineticola* End. is mentioned as a predator of the mite. The larva of the species with which they were concerned is illustrated and agrees in general with the larva found in Essex, but the illustration is small and the antennae are shown as consisting of five joints. No indication is given of the means of identification of this insect, or of the fact that the identity of the larva figured by them was unknown. Correspondence with V. Kanervo, one of the authors of the paper, has revealed that little is known by them about the species or characters of the larva. J. Listo, who carried out the biological work and drew the figure, has since died and left no further information on the subject, but it is probable that only the adults were considered for identification purposes.

The larvae of the two forms are not mentioned elsewhere in the literature, but while the larvae found in Essex and Kent could not be identified from published descriptions or figures, it was clear that they were consistently distinct from *C. psociformis*, differing from it in shape, size, colour and antennal dimensions. Moreover, the resulting adults bred true, the larvae of successive generations were identical, and even the eggs were appreciably different from those of *psociformis*. These striking differences in the immature stages of the two forms confirm the view that *C. pineticola* and *C. psociformis* are distinct species, despite the close similarity of the adults.

Immature Stages and Bionomics of Conwentzia pineticola **End.**

Egg.

The egg (Plate XIX, fig. 1) averages 0·44 × 0·23 mm. It is elongate-oval in form, similar to that of *C. psociformis* but slightly smaller ; it has the usual micropylar projection at the anterior pole. The colour is salmon-pink, gradually darkening as the time of hatching approaches. When the female has laid an egg it moves the abdomen from side to side over it from one end to the other, depositing all over it a white, mealy layer which is just visible, but which does not extend on to the leaf. In *C. psociformis*, this deposit is far more conspicuous although the egg is white, and, in addition to covering the egg, it is usually deposited quite thickly on the leaf or twig surface around the egg. The secretion appears similar to the wax which coats the surface of the wings and body of the adults.

The eggs are laid singly on the leaves or bark of fruit trees. They are usually placed on the under surface of the leaves, not at the margins, whereas those of *C. psociformis* are usually deposited at the margins.

Larva.

The newly hatched larva is 0·8 mm. long. It is transparent and colourless except for the red eyes, but as soon as food is taken the gut shows clearly through the body wall. The head at this stage is relatively large, and the ratio of the length of the

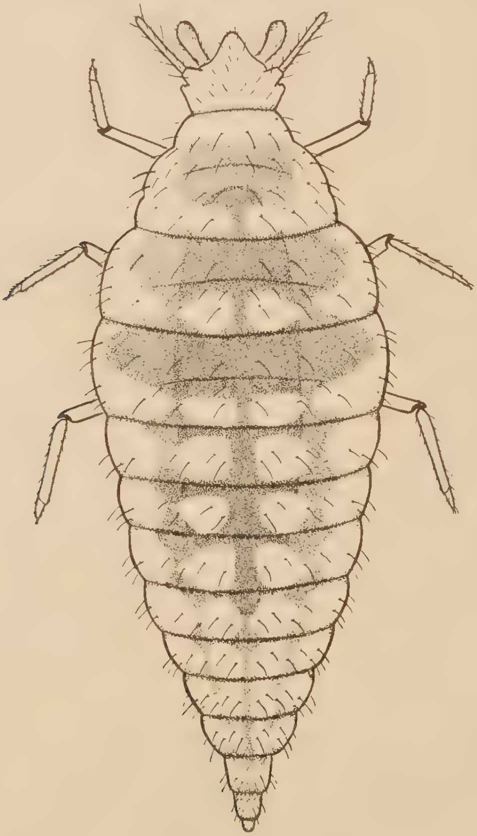

Fig. 2.—Third-instar larva of *Conwentzia pineticola* End.

antennae to that of the palpi is similar to that of the fully grown larvae. The second antennal joint is 3·7 times as long as the basal joint.

There are two moults before the larva becomes fully grown. The third-instar larva when fully grown is approximately 3·4 mm. long (fig. 2). It is relatively much shorter and broader than the fully grown larva of *C. psociformis* (fig. 3) and has shorter legs and antennae. The head is small and partly retracted into the prothorax ; it is pale in colour, the antennae and palpi are translucent and the distal segment of each

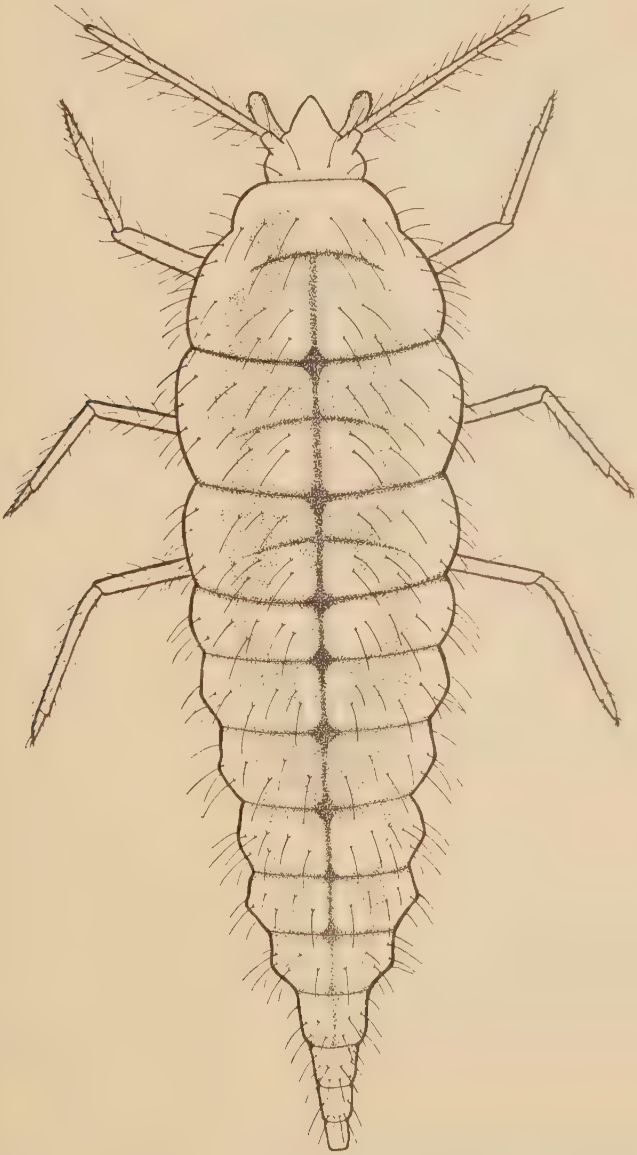

Fig. 3.—Third-instar larva of *Conwentzia psociformis* (Curt.).

palp is clothed with very fine white hairs. The distal segment of the antenna is approximately three times as long as the basal segment ; the antennae, which are 0·34 mm. long and only slightly longer than the palpi, are clothed with fine white hairs, very much shorter than in *C. psociformis.*

TABLE II.

Comparison of the third-instar larvae of *C. pineticola* and *C. psociformis*. (Averages in millimetres for 25 larvae of *pineticola* and 20 of *psociformis*.)

	C. pineticola	*C. psociformis*
Width of head ...	0·35	0·35
First antennal joint...	0·08	0·09
Second antennal joint	0·26	0·80
Labial palp	0·25	0·25
Length of 3rd tarsus + tibia	0·41	0·68

m The ratio $\frac{\text{length 2nd antennal joint}}{\text{width of head}}$ is 0·74 in *C. pineticola* and 2·29 ins. *C. psociformis*.

The two setae that project over the tarsal claws are fine and tapering, and shorter than the body setae ; in the genus *Coniopteryx*, they end bluntly, and in *Semidalis aleyrodiformis* (Steph.) they are fine, curved and tapering, but about equal in length to the body setae. The body is clothed with white setae, very much shorter than in *C. psociformis*, but arranged in a similar pattern ; the setae arise from small tubercles. The legs bear shorter hairs than the body. In general appearance, the larva is deep orange-red with white markings, whereas that of *C. psociformis* appears white with brownish markings. The body contents in both species are deep red or brown ; the white areas, more extensive in *C. psociformis* than *C. pineticola*, are the fat body seen through the body wall, and the pattern thus formed is similar in the two species, though very variable. In *C. pineticola* the body is white laterally, and the white area extends across the anterior end of the prothorax. Each segment has a pair of white areas dorsally, which in some forms are continuous with the lateral white bands, but they never coalesce medially. In addition to the general red colour of the body, the gut often shows as a central, darker red area.

The terminal abdominal segment of the larva secretes a sticky substance which is used, especially when the larva is young, as a means of attachment. At the end of the larval life, the eversible anal papilla is used as a spinneret to construct the cocoon. According to Withycombe (1923), the silk is secreted by the modified Malpighian tubules. The feeding habits of the larva are described under a separate heading below.

Pupation.

When fully fed, the larva spins a cocoon and rests within it as a prepupa. The cocoon is circular and about 4 mm. in diameter, except when formed in crevices of the bark, when it may be irregular in shape (Pl. XIX, fig. 2). It is a double structure, consisting of an outer and a smaller, inner envelope, and the latter usually extends both above and below the prepupa. The cocoons are pure white and very conspicuous. In summer, they are usually spun on the leaf surface and occasionally on the twigs, but in the autumn they are constructed for overwintering on or under the bark of tree trunks.

Life history.

Emergence of the adults from the overwintering cocoons normally takes place during April, but if cocoons are kept indoors at a higher temperature emergence can be obtained much earlier. Adults are present from April until November, there being more than one generation a year. Eggs are laid in April, May and June by the females which emerge from the winter cocoons, and eggs of the second generation are laid mainly in July and August. The length of life of the females, however, is long, so that there is considerable overlapping of generations, all stages being found together from May until November. There is a partial third generation, and in some years this reaches large proportions in orchards in south-eastern England. Adults of the second generation commence to oviposit at the end of July, and their eggs give rise to third generation adults within about three weeks. In 1947, very large numbers of *C. pineticola* occurred in orchards during late August and September. Adults of the third generation, and probably some of the later members of the second, were active in the field and continued to oviposit until November ; consequently larvae were active in mid-December. This occurred again in 1949.

The duration of each stage varies considerably according to weather conditions, the time from oviposition to emergence of the adult from the cocoon ranging from a minimum of 16 days in mid-summer to a maximum of 36 days in September (Table III). The winter is passed in the prepupal stage and pupation of the overwintered generation occurs in March ; adults emerge two or three weeks later.

TABLE III.

Duration of immature stages of *Conwentzia pineticola* End. in 1949.

Stage		Number of days		
		June–Aug.	Sept.	Oct.
Egg	range	4–7	6–9	7–19
	average ...	5	7	12
Larva	range	6–8	8–15	12–23
	average ...	7	10	16
Cocoon	range	6–8	10–12	—
	average ...	7	11	—
Egg-adult	average ...	19	28	—

For copulation, the male approaches the female from behind and thrusts its head and thorax beneath the female's abdomen, clinging to its hind coxae by means of the mouthparts and fore-legs ; the abdomen of the male is curved upwards to meet that of the female. The female sometimes walks about, dragging the male in this position, and if disturbed may even take to flight. Copulation lasts from 15 to 30 minutes.

In the laboratory, the male frequently lives for more than four weeks, and the female may live up to eight weeks ; under natural conditions they probably live even longer. The female lays as many as eight eggs per day on the average in July and August. The highest number of eggs laid by one female under laboratory conditions was 107, laid over a period of seven weeks from September 12th to October 29th, 1949. An average of five a day was laid through part of September, but during October the rate decreased. When about to oviposit, the female moves rapidly in a frenzied fashion over the leaf or twig, apparently testing the surface with the palpi ; it stops suddenly and oviposits within a few seconds, and finally deposits over the egg the layer of wax already described. The feeding habits of the adult are described below.

Occurrence.

C. pineticola is partially gregarious. Usually, in any particular locality, it is either present in large numbers or is apparently entirely absent. According to

Withycombe (1923), this applies to *C. psociformis* also, and he suggests that the variation in certain morphological characters may be due to the isolation of strains.

C. pineticola has been found in Essex solely in association with outbreaks of the Fruit Tree Red Spider Mite. It has been found on apple, pear, plum, damson, cherry and walnut, and very occasionally on neighbouring hedgerow plants. No alternative habitat has been found. Its occurrence in orchards is by no means consistent. In 1947, it was numerous from August onwards in many orchards with high red spider mite infestations, but there were certain orchards with high mite populations where it did not appear. Very large numbers of cocoons were present on the trees in the autumn, yet in the following spring and summer the species was absent. Orchard spraying practices account for the death of many individuals in their cocoons, but by no means all. Withycombe (1924) explains a similar phenomenon in *C. psociformis*, which he found to be always exterminated by a parasite, *Lygocerus* sp., in the second year. However, of a large number of *C. pineticola* cocoons observed in the laboratory, only a small percentage were parasitised ; the rest gave rise to adult insects which did not survive in captivity although they lived for a time feeding on red spider mite winter eggs. It appears that the adults do emerge in the spring but do not remain in the orchards, where the only available food in April is the winter eggs of the mite. In 1948, when the summer was cool and damp, very few specimens were found, but in 1949, large numbers again appeared late in the summer. This sudden appearance is due to the influx of adults, presumably of the second generation, into the orchards in late July. Listo, Listo and Kanervo (1939) mention the same spasmodic occurrence of the species in orchards of Finland.

Conwentzia psociformis (Curtis).

In Essex, this species has been found in small numbers on fruit trees and also on other trees and bushes, particularly hawthorn. In Kent, it was found on *Buxus* at East Malling ; Killington does not mention Kent in his account of its distribution.

A good account of it is given by Killington and the larva is figured both by him and by Withycombe (1923). Certain details of its morphology as compared with that of *C. pineticola* have already been considered. Withycombe, when discussing the two forms, *C. pineticola* and *C. psociformis*, gives the length of the larval antenna of the latter as 0·75 mm., whereas the present author finds it to average 0·9 mm. The life history as revealed in the present investigation conforms to that described by Killington ; there are two generations a year, and the insect does not remain active after September.

Feeding habits of Conwentzia **species.**

An early reference to the feeding habits of *C. psociformis* is by Schlechtendal (1882), who describes it as a parasite of spider eggs ; he evidently mistook the Coniopterygid cocoon for the egg cocoon of a spider. According to Killington, *C. psociformis* feeds on a variety of Aphids, Coccids and mites ; Withycombe (1924) says that it sometimes affords a valuable check to *Phylloxera punctata* Licht. on *Quercus* and also to *Paratetranychus quercinus* Berl. Observations in Essex confirm this range of food, but in orchards the main food is the Fruit Tree Red Spider Mite.

C. pineticola feeds in orchard conditions almost entirely on mites, and this is true of both larval and adult stages. In captivity, the larvae feed on each other if their normal food is scarce, and larvae have been observed in the field feeding on eggs of their own species. The larvae pierce red spider mites with their jaws and suck out the contents, leaving the empty skins on the leaf ; mite eggs also are sucked dry, the complete empty shells remaining. The larval mandibles are short and almost straight, and the maxillae bear backwardly-directed barbs ; the suction channel is formed by the mandibles and maxillae together. The adults pick up the mites and completely devour them, and bite off the top halves of the eggs and take most of the contents, leaving behind the lower halves of the shells. When hunting for food, they move quickly and at random over the leaf, feeling the surface with their palpi. It is only when actual contact occurs between the palpi and the mite that the latter is noticed ; contact with the tarsi gives no response, and vision seems to play no part. Both the larvae and adults show a preference for adult female mites, but all stages are taken. Even the newly hatched larvae, which are scarcely larger than the adult mites, will attack and suck female mites. Adults have been observed to devour as many as 30 adult female mites in an hour ; much of this time is spent in searching and in cleaning the mouth parts with the fore-legs, a process which is repeated frequently. Daily counts show that the average daily consumption per larva is 15–20 mites, and that the corresponding figure for third-instar larvae is 30–35. The adults feed somewhat spasmodically, but consume 30–40 mites per day. The numbers consumed depend upon the stage of mite that is being attacked. In the autumn, larvae, and occasionally adults, are still active in the field after the leaves have fallen and when only eggs of the mite remain. Under these conditions, they live entirely on winter eggs. Where the infestation of mites has been severe and the winter eggs have been laid in a series of layers among sheets of webbing, *C. pineticola* larvae are often trapped within these layers ; they spend their whole life in a small area and eventually spin their cocoons in the webbing.

Other Coniopterygids.

Two other Coniopterygid species are found feeding on red spider mites where infestations are severe in orchards. These are *Coniopteryx tineiformis* Curt. and

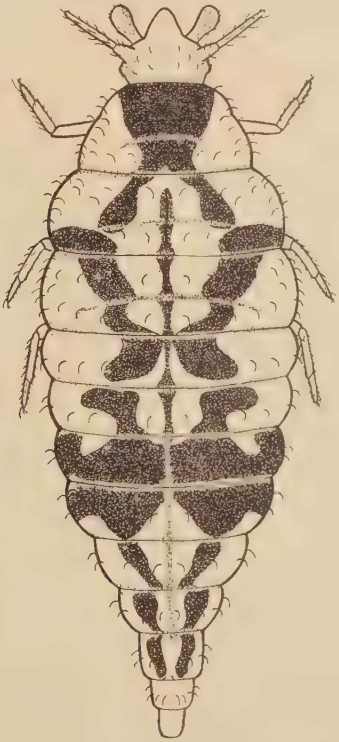

Fig. 4.—Third-instar larva of *Semidalis aleyrodiformis* (Steph.).

Semidalis aleyrodiformis (Steph.), both of which are described by Killington. The larva of the latter (fig. 4) is very distinct because of its conspicuous black and white coloration, but its shape and proportions are very similar to those of *C. pineticola*. The feeding habits of these two species are very similar to those described for *Conwentzia*. Neither is abundant on fruit trees, but they are more plentiful in well tended than neglected orchards. Both occur on other plants, including hawthorn, sloe and oak.

Summary.

Conwentzia psociformis (Curt.) and *Conwentzia pineticola* End. differ as adults in only two characters, and since there are variable and intermediate forms occur, reliable determination is sometimes impossible. In the past, these two species have often been regarded as two forms of the same species.

The immature stages of *C. pineticola* are, however, very different from those of *C. psociformis* and show that the two are distinct species and can readily be separated on larval characters. The larval stages and egg of *C. pineticola* are described for the first time, and an account of its life history given.

The feeding habits of these two species are described, and mention is made of two other Coniopterygid species that occur in orchards.

Acknowledgements.

The author thanks Dr. A. M. Massee for his help in this investigation, and Miss E. C. Thompson for the photomicrographs.

Dr. F. J. Killington and Prof. V. Kanervo have both very kindly given their help in this problem. The author is grateful to the Agricultural Research Council under whose grant the earlier part of the work was carried out.

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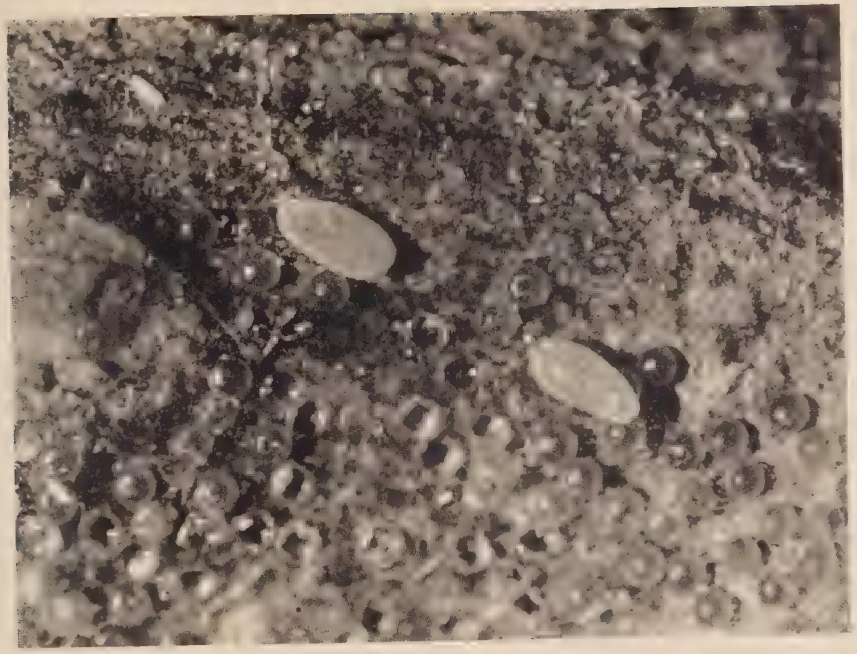

FIG. 1. Eggs of *Conwentzia pineticola* End. on surface of twig with winter eggs of the red spider mite.

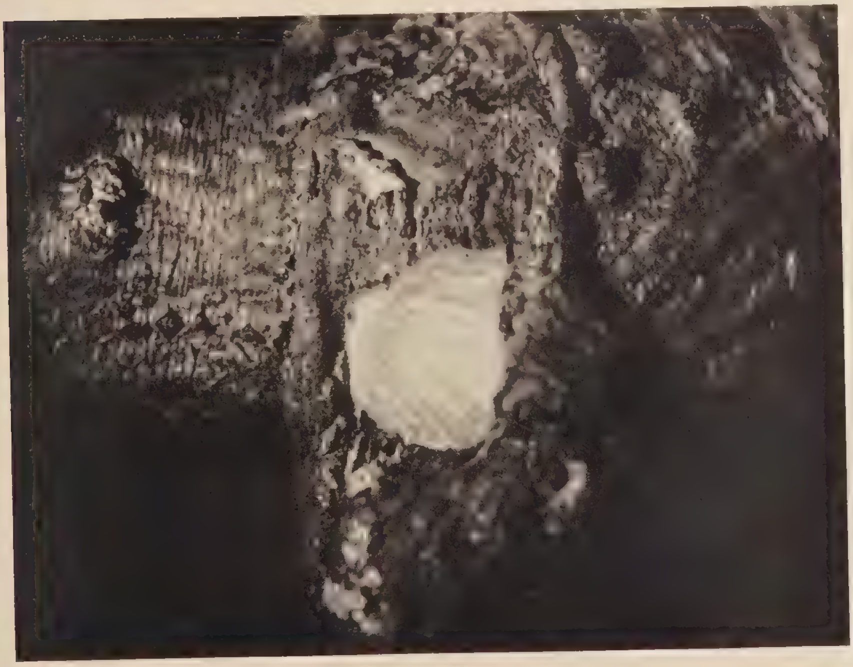

FIG. 2. Cocoon of *Conwentzia pineticola* End.

A NEW GALL MIDGE (DIPT., CECIDOMYIDAE) PREDACEOUS ON THE FLOUR MITE, *TYROGLYPHUS FARINAE* (Deg.).

By H. F. Barnes, M.A., Ph.D.

Rothamsted Experimental Station.

Owing to the courtesy of Miss H. Dombrowski (E. V. Dombrovskaya) of the Zoological Institute of the Academy of Science, Leningrad, U.S.S.R., it is now possible to describe a sixth species of gall midge belonging to the genus *Trisopsis* Kieffer. Males, females and larvae of this species were received from Russia in April, 1937. The larvae had been found during the previous winter preying on the Flour Mite, *Tyroglyphus farinae* (Deg.) during laboratory observations on the predaceous mite, *Cheyletus eruditus* (Schr.), which had been kept with the flour mites in paraffin wax cells at the Institute for Plant Protection, Leningrad (Sorokin, 1938).

Trisopsis tyroglyphi sp. n.

Male.—Length about 1 mm. Three compound eyes, the median-dorsal consisting of the dorsal third of the two normal compound eyes, the latero-ventral ones being the basal third of each. Antennae : flagellar segments slightly more than one and a half times as long as thorax and abdomen without genitalia, 2+12 ; basal segment transverse, low cup-shaped, second globular ; first and second flagellar segments fused, basal nodes globular, distal nodes slightly elongate ; third flagellar segment with basal node subglobular, slightly wider than long, stem about twice as long as broad and about as long as basal node, distal node globular, as wide as long and about as wide as basal node, neck about twice as long as broad, about as long as distal node, basal node bearing one circumfilum with about seven loops reaching about half-way up stem and ring of stout setae, just over twice as long as loops, one seta between each point of attachment of circumfilum, distal node bearing basal circumfilum with about six to seven loops reaching half-way up node and distal circumfilum with about seven loops reaching almost to node of next segment, with ring of stout setae, about twice as long as loops, one seta between each point of attachment of distal circumfilum ; tenth flagellar segment almost the same proportions and length, but stem and neck are slightly narrower, about three times as long as broad ; terminal segment with globular basal node, stem slightly longer and about four times as long as wide, distal enlargement about equal in length to basal node plus stem and about twice as long as wide. Palpi : short, three-segmented, proximal segment slightly smaller and more quadrate than other two. Wings : hyaline, slender basally, subcosta joining costa just beyond basal slender part of wing, third vein interrupting margin at apex, fifth vein simple, bending to join margin as though branched. Claws simple, slender, moderately curved, pulvilli shorter. Genitalia : basal clasp segment slender, slightly curved ; terminal clasp segment slightly swollen at base ; dorsal plate with deep wide V-shaped emargination, lobes rounded, slightly longer than wide ; ventral plate broad, rounded, about as long as dorsal lamellae ; style long, tapering, reaching just beyond basal clasp segment.

Cotypes, Cecid. 3091–7 (in the Barnes collection).

Female.—Length about 1 mm. Eyes as in male. Antennae : 2+12, first and second flagellar segments fused, basal enlargements cylindrical, that of third flagellar segment nearly twice as long as broad and about twice as long as neck which is slightly more than twice as long as broad ; that of tenth flagellar segment about half as long again as broad, about twice as long as neck which is slightly more than twice as long as broad ; distal segment about two and a half times as long as broad. Ovipositor short, lateral lamellae nearly oval, ventral lamella small. Otherwise about as in male.

Cotypes, Cecid. 3098–3102 (in the Barnes collection).

Larva.—Typical predatory form because of head capsule, nine pairs of pseudopods, no breastbone and long setae. Anal segment with four long setae distally.

Cecid. 3178–80.

Other specimens of this consignment were returned to Miss H. Dombrowski.

Locality.—Laboratory mite cultures, Leningrad.

Habit.—Predaceous on *Tyroglyphus farinae* (Deg.).

One point of interest concerning *T. tyroglyphi* is that it is the first *Trisopsis* species whose larvae are definitely known to feed on mites, although it is probable that all the species are at least predatory. The type of the genus, *T. oleae* Kieffer 1912, was described from both sexes from Wellington, S. Africa, where the larvae were found living in the fruits of *Olea verrucosa*. The second species to be described was *T. alluaudi* Kieffer 1913, a female of which was caught in a forest in Kenya. The third species is *T. bifida* Brèthes 1915, described from a single male caught at Buenos Aires. The fourth species, *T. hyperici* Tavares 1919, was described from a female reared from the galls of *Geocrypta hypericina* Tavares found at Redondela (Pontevedra), Spain. The fifth species, *T. hibisci* Felt 1935, described from both sexes, was reared from the seed pods of *Hibiscus militaris* at Tallulah, Louisiana, U.S.A., by R. W. Harned during August, 1933.

A second point of interest is the peculiar separation of the compound eyes into three parts. The only other Cecidomyid genus in which this occurs is *Triommata* Barnes. The type and only species is *T. coccotroctes* Barnes 1931, described from Sierra Leone. Its larvae are known to be predaceous on mealy-bugs.

The use of a gall midge in the biological control of a mite has been suggested by Elago (1940). He experimented with *Silvestrina tyrophagi* Dombrowski 1940 and concluded that it gave promise in the control of *Tyrophagus castellanii* (Hirst) (syn. *T. noxius* Zachvatkin) in hemp seed. He claimed that the number of mites in about half a ton of infested seed into which eggs, larvae and adults of *S. tyrophagi* had been introduced was reduced by 78·5 per cent. after a month.

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FURTHER TOXICOLOGICAL STUDIES ON SPECIES OF *CAPNODIS* IN ISRAEL.

By E. Rivnay.

The toxicity of the fumes of certain substances to the larvae of *Capnodis* was dealt with in an earlier paper (Rivnay, 1947). A brief reference was also made in this paper to the possibilities of contact insecticides against the neonate larvae and to the effect of certain stomach poisons upon the adult beetle.

The status of the pest is far less acute than it was several years ago on account of the spraying of the trees with stomach poisons and the introduction of irrigation into many plum orchards. Since 1947, however, some of the new synthetic insecticides have become available and it was thought desirable to test some of these to ascertain whether the present control measures could be improved upon by making them simpler, cheaper and, if possible, safer. To this end, the effect of DDT and BHC was tested against *Capnodis* when applied to the trees as a dust and as a spray. The same insecticides were also tested as contact poisons in the neonate larvae against which other substances had failed. Ethylene dibromide, which proved so effective, has to be applied with great care to avoid damage to the roots of the trees. BHC is known to have a fumigant action and this suggested that it would be worth trying this substance against the larvae boring in the roots.

The Effects of Fumes of BHC upon Boring Larvae.

Tests to determine whether the fumes of BHC are effective against *Capnodis* larvae were made in three ways :—

(1) Eight pieces of branches containing 50 one-day-old larvae were placed close to a petri dish, filled with 3·5 per cent. BHC* dust in a gallon jar. The jar was closed tightly, and the larvae allowed to remain in it for five days when the bark was peeled off the twigs and the mortality of the larvae assessed. This experiment was carried out twice ; on the first occasion 40 live larvae were discovered and on the second, 23 ; no dead larvae were found. In the controls, 32 and 26 live larvae were found respectively.

(2) In a second series of tests, pieces of an almond branch, in which 30-day-old larvae were boring, were buried 30 cm. deep in the ground. Three bundles of such pieces of wood were used in each trial. One bundle was dusted with 20 grams of 5 per cent. BHC dust, and then covered with earth ; the second bundle was covered with earth over which 6 cc. ethylene dibromide was applied ; the third bundle was covered with earth only, as a control. Five days later the bundles were taken out and the mortality of larvae assessed. These tests were repeated three times. Table I presents the mortality in the three trials.

(3) Another trial was made in the field. Fifteen-year-old plum trees, suspected of being infested with *Capnodis*, were chosen for this purpose. The roots were uncovered and a 5 per cent. BHC dust was applied thickly over the roots, which were then covered with earth. Five trees were treated with 150 grams each, while five other trees were treated with 300 grams of the dust. Twenty days later, the roots were uncovered and mortality amongst the boring larvae assessed.

In the trees treated with 150 grams, one larva, three pupae and one adult were found in the roots, all alive. In the trees treated with 300 grams, five larvae, and one adult were found alive in the roots ; one larva close to the surface and one adult outside the roots were found dead. The larva was found very close to the surface of the root which was well covered with the dust. The beetle presumably came in direct contact with the dust on its way to the surface.

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*Throughout this paper BHC is to be taken to mean technical benzene hexachloride containing about 13 per cent. of the gamma isomer.

TABLE I.

Mortality of larvae due to fumes of insecticides.

	BHC		Ethylene dibromide		Control	
	Live	Dead	Live	Dead	Live	Dead
First trial	20	3	0	26	10	18*
Second trial	38	0	0	23	14	2
Third trial	55	3	0	41	56	0
Total	113	6	0	90	80	20
Percentage Mortality ...		5 per cent.		100 per cent.		20 per cent.

*Death probably due to excessive moisture.

From these three series of experiments, is is obvious that the fumigant action of BHC is ineffective against the larvae boring within the roots.

The Effect of Contact Insecticides upon Neonate Larvae.

The writer (Rivnay, 1947) considered that the possibility of finding some dry chemical substance, that would kill neonate larvae by contact and yet be harmless to the tree and soil, was not particularly promising. Later, when some of the newer insecticides became available this view became modified.

There is one difficulty in obtaining exact information from toxicity trials with *Capnodis* larvae, which generally live for only two or three days under conditions of room temperature and humidity. The effect of the poison may only be noticed after three or four days in which case the death of a larva may not be due to the toxicity of the poison. Consequently, one cannot assess the toxicity to the larvae by simply bringing the insecticide into contact with them. Furthermore, the neonate larvae are wriggling constantly, and one is never certain whether this behaviour is normal or a reaction to the poison. It was therefore imperative in all cases to assess the mortality of the larvae by placing them, after treatment, in a slit in the wood. It was assumed that those which penetrated and began to feed were not affected by the poison ; those which were affected, although wriggling constantly, could not penetrate into the wood. This was the basis on which the results of the two following series of experiments were assessed.

The insecticide was diluted with sand at the rate of one to nine and a small quantity of the mixtures sprinkled into a petri dish. Newly hatched larvae were allowed to crawl or wriggle upon the layer in the dish for an hour, after which they were removed and placed in slits in pieces of wood.

The following insecticidal powders were mixed with sand : 3·5 per cent. BHC ; 5 per cent. BHC ; 5 per cent. DDT ; and a mixture of 1 per cent. chlordane and 3 per cent. Thanite†.

The experiments were repeated as shown in Table II. It should be noticed that not all the larvae were recovered in the controls ; many perished because of excessive moisture or gum exudations. For this reason, the rate of survival is considered and not the mortality rate.

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†Believed to be mainly isobornyl thiocyanoacetate [Ed.]

TABLE II.

Survival of larvae after 60 minutes' contact with various insecticides.

Name of Insecticide	No. of larvae employed	No. of trials	No. of larvae recovered	Per cent. survival
BHC 3·5 per cent.	130	4	4	3
BHC 5 per cent.	25	1	0	0
DDT 5 per cent.	115	2	33	28·6
Chlordane 1 per cent. and Thanite 3 per cent....	100	2	0	0
Control	205	5	132	64·3

The above results indicate a very low survival rate in the case of the BHC and chlordane-Thanite mixture after a limited period of contact but it was also important to known how long these insecticides remained active in the ground before they lost their effectiveness. This information is necessary to determine how often an application of this nature should be repeated in order to protect the roots of trees against *Capnodis* larvae. Samples of these mixtures were, therefore, put into small bags made of loose cloth and buried in the ground at a depth of about 10–15 cm. After various periods of time, one bag of each mixture was removed from the ground and tested as in the previous experiment. The results after periods of 20, 30 and 50 days in the ground are presented in Table III.

TABLE III.

Survival of larvae after 60 minutes' contact with insecticides which had been in the ground various lengths of time.

Name of Insecticide	Period in the ground	No. of trials	No. of larvae	No. of survivals	Per cent. survivals
BHC 3·5 per cent.	20 days	2	50	2	4
DDT 5 per cent.	20 ,,	2	50	23	46
Chlordane 1 per cent. and Thanite 3 per cent.	20 ,,	2	50	13	26
Control	—	2	50	30	60
BHC 3·5 per cent.	30 days	2	45	4	8·8
DDT 5 per cent.	30 ,,	2	45	33	73·3
Chlordane 1 per cent. and Thanite 3 per cent.	30 ,,	2	45	19	42·2
Control	—	2	45	25	55·5
BHC 3·5 per cent.	50 days	2	40	4	10
Control	—	1	20	15	75

It is clear from the above that BHC, DDT, and the chlordane-Thanite mixture deteriorate to some extent with time. BHC retained its effectiveness up to the end of the 50-day period, but the other two had lost theirs after 30 days and the experiment was, therefore, continued without them.

The conditions under which the previous experiments were carried out were quite foreign to the mode of life and activity of *Capnodis* larvae. As a rule the neonate larvae never remain on the surface, but penetrate into the ground immediately after hatching, heading towards the roots which they detect by the odour (Rivnay, 1945). Further experiments were therefore designed in which the larvae had to wriggle their way through the layer of insecticide on their way to the root ; the layer of insecticide remained intact throughout the period of trial, and yet was subject to the effects of rain, sun, heat, etc. ; the trials could be carried out at desired intervals.

A wooden box 45 cm. high, with four vertical partitions, was buried in the ground. At the bottom of each compartment two layers of sand 10 cm. thick had between them a layer of 2–3 cm. of sand mixed with an insecticide dust. In each compartment, a different insecticide dust was employed at the rate of one part to nine parts of sand.

The insecticides used in the 10 per cent. mixtures were BHC 5 per cent. dust, methoxychlor 5 per cent. dust and 5 per cent. dieldrin. One compartment was left without insecticide as a control. At a given date, a sample of each of the sandwiched layers was taken in a tin tube by pushing the tube perpendicularly into the moistened sand and removing it with the column of the sandwiched layers intact. Small pieces of almond branch, about 12 to 15 mm. in diameter and about 15 mm. long, were pushed into the sand at the bottom end of the tube, and some neonate larvae were placed on the upper layer of the sand. Soon the larvae penetrated the sand, and guided, perhaps, by the odour of the almond twigs they headed towards them but they had to pass through the layer of insecticide before the twigs could be reached. If many larvae were able to reach the pieces of almond twig and to penetrate into them, it was a sure sign that the insecticide through which they had penetrated was of little value. The results of these trials are given in Table IV.

TABLE IV.

Survival of larvae after passing through layers of sand containing insecticides.

Date samples were taken	25.viii.48	30.viii.48	8.ix.48	16.ix.48	23.ix.48	20.vi.49
Number of days sample had been in ground ...	28	33	42	50	57	327
Number of larvae employed in each case ...	75	20	40	50	35	43
Survivals with :						
BHC 5 per cent. dust...	0	0	0	0	0	0
Dieldrin 5 per cent. ...	0	0	0	0	0	0
Methoxychlor 5 per cent. dust	10	0	0	0	0	0
Control...	24	3	6	7	12	8

It will be noticed that the survivals in the control are quite low, from 15 to 34 per cent. only. This may be due, either to excessive moisture, or to the compactness of the soil, which made it difficult for the larvae to penetrate. The trials show, however, that even a year after burial the three insecticidal dusts remained active, and prevented the larvae from infesting the roots.

An experiment was carried out in which the tin tube was replaced by a glass tube in an endeavour to determine whether the larvae were repelled by the insecticidal dust and refused to wriggle further or whether they were actually killed by contact with the dust.

A layer, 25 mm. deep, of 5 per cent. methoxychlor dust mixed with sand in the proportion of 1 to 9 was introduced into a test tube open at both ends between two layers of sand of similar thickness. At one end of the tube, pieces of almond twigs were placed and at the other 25 larvae were allowed to wriggle into and through the layers of sand. After three days, 12 of the 25 larvae were found to be alive in the pieces of almond twig in the control tube, but, in the tube containing insecticide, 17 larvae were seen at various depths. Many of these succeeded in reaching the last layer of sand but died before reaching the wood ; one even reached the wood, but died before it could begin to gnaw its way into it. Many others were found dead in the toxic layer. From this it was clear that the insecticide did not repel the larvae.

It was also necessary to ascertain what effect these dusts had upon the roots of the trees themselves. In an experiment carried out at Hedera in 1947, 150 and 300 grams of BHC 3·5 per cent. dust were applied to the ground above the roots. The trees were examined three months after the application was made and no indication of ill effect was noticed.

Another test was made at Rehovot in 1949. Eight 6-year old plum and peach trees were treated with either 100 grams of 5 per cent. BHC or 5 per cent. methoxychlor dust. The insecticide powder was mixed with 900 grams of sand, strewn around the trees at a depth of 5 cm. and then covered with earth. No ill effects have been noticed up to the time of writing.

Experiments to Assess the Toxicity of Synthetic Organic Insecticides on Adults.

Experiments to assess the toxicity of the synthetic organic insecticides to adult *Capnodis* were made as early as 1945 and were continued on subsequent occasions. The purpose was, as mentioned earlier, to see whether the inorganic insecticides which are now in use could be replaced by poisons less toxic to the trees or human beings.

The tests were made by feeding adult *Capnodis* upon almond twigs dipped in the dust or a suspension of the insecticide. Where necessary, a spreader or sticker was added to the suspension. The beetles were introduced to the twigs one hour after the insecticide had been applied and throughout the experiment the same type of twigs were used. The results of these experiments, carried out in 1945, in which very small doses of the insecticides were employed, are presented in Table V. They show that most of the beetles lived for quite a long time after consumption of twigs treated with DDT, a large percentage surviving even after 30 days, whilst those fed on arsenate-treated twigs died much sooner.

TABLE V.

Mortality of adult beetles after feeding on various insecticides.

(Laboratory Test)

Name of insecticide	No. of beetles	No. of tests	No. of dead beetles after period of :						No. Survived	Per cent. survival
			2 days	4 days	8 days	12 days	16 days	30 days		
Lead arsenate 0·5 per cent spray	25	2	7	15	3	0	0	0	0	0
Cryolite 0·7 per cent. spray...	40	3	0	6	0	24	3	7	0	0
Barium fluosilicate 0·7 per cent. spray ...	20	2	0	0	4	3	4	9	0	0
DDT 1 per cent. dust	39	3	0	6	9	0	6	3	15	38
DDT 0·5 per cent. spray...	39	4	0	5	2	5	5	9	13	33
DDT 0·75 per cent. spray...	35	4	0	4	2	2	1	8	17	48
Control	25	2	0	1	0	0	1	0	23	92

Further experiments were carried out in 1947 and 1949 with larger doses of these compounds. These tests showed that some synthetic compounds, when applied at higher concentrations, might kill the beetles as quickly as cryolite. The use of these compounds against other insects had showed, however, that the value of the synthetic insecticide depended greatly upon its stability under outdoor conditions. Consequently, experiments were carried out in 1950 with substances which were allowed to weather from one to twelve days. The trees were sprayed and after a given time beetles were placed on a branch over which a sleeve was placed. These experiments indicated that, when applied as dusts, the organic compounds lose their toxicity much too soon but, as suspensions of wettable powder, they retain it for at least 10 to 12 days. The results are shown in Table VI.

TABLE VI.

Mortality of adult beetles after feeding on trees sprayed with various insecticides.

Name of Insecticide	No. of beetles	No. of tests	No. of dead beetles after period of				No. escaped	No. survived	Per cent. survival
			2 days	4 days	6 days	8 days			
Cryolite dust	35	7	9	11	6	3	0	6	17
Cryolite 1 per cent. spray ...	45	8	5	9	6	2	0	23	51
DDT 5 per cent. dust... ...	45	8	5	2	6	2	12	16	36
DDT 10 per cent. dust ...	45	8	4	4	6	4	7	20	44
DDT 0·5 per cent. wettable spray	30	6	5	4	5	1	4	11	37
BHC 5 per cent. dust ...	10	2	3	1	0	0	1	5	50
BHC 0·5 per cent. wettable spray	15	3	9	0	0	2	3	1	7
Methoxychlor 0·5 per cent. wettable spray	45	8	9	10	5	3	1	17	38
Control	30	6	0	1	1	0	0	28	93

Mortality comparable to that with cryolite was obtained by a spray which contained 0·5 per cent. of BHC wettable dust.

Practical Considerations.

When it is suggested that an insecticide or a method of control that has been in use for some time should be replaced by a new one, it is necessary to consider whether or not the proposed change is economically sound.

It is not unlikely that in certain localities, the labour involved in applying an insecticidal dust around the trees would cost more than the spraying of the trees with insecticides. In such cases, the disadvantage of the greater cost of the dusting must be weighed against that of the danger resulting from the presence of residues of the spray on the fruit.

In the case of the spraying of trees against adults of *Capnodis*, it is not suggested that an expensive insecticide should entirely replace a cheap one that is already widely used, but rather that if a comparatively expensive synthetic poison is employed against other pests, *Capnodis* also will eventually be controlled, or *vice versa*. When it is desired to control two pests one of which is susceptible to a contact poison only, and the other to either a contact or a stomach poison, it may be economical to use a more expensive insecticide to control both pests at the same time.

Summary.

Whilst the status of *Capnodis* as a pest in Israel has been considerably reduced by the control measures that have been adopted, it was considered desirable to test the new synthetic contact insecticides, in particular DDT and BHC.

Techniques for the determination of the effect of insecticides on neonate larvae of *Capnodis* are described and the results discussed.

It is shown that BHC and DDT are ineffective as fumigants against the boring larvae.

Used as a contact insecticide against the larvae, a high degree of mortality is obtained with 5 per cent. BHC over a period of 50 days. DDT is far less effective. BHC, dieldrin and methoxychlor remained effective for over a year if left undisturbed in the ground.

Against adult *Capnodis*, BHC gave results comparable to those obtained with cryolite and retained its activity for at least twelve days after application.

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STUDIES OF THE RESPONSES OF THE FEMALE *AËDES* MOSQUITO. PART IV. FIELD EXPERIMENTS ON CANADIAN SPECIES.*

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(Plate XX.)

Laboratory experiments (Brown, Sarkaria & Thompson, 1951 ; Peterson & Brown, 1951) have already shown the importance of moisture, carbon dioxide and warmth as factors attracting the female yellow-fever mosquito, *Aëdes aegypti* (L.) to bodies. This paper reports the results obtained by similar studies made under field conditions, involving several species of *Aëdes* that frequent the Canadian forest. The investigations were made by means of a pair of robots so constructed, heated and clothed as to constitute dummy men.

Apparatus.

Each robot (Pl. XX) consisted of a stainless steel tank, 24 ins. high, elliptical in cross section with the longest and shortest diameters 15 and 10 ins. respectively, and holding 100 lb. of water warmed to body temperature. It rested on a metal stand the height of which, when spiked into the ground, was 26 ins. A metal dish inserted in the lid of each tank carried an electric motor for stirring the water, and the necessary electrical connections for a thermostat and a 125-watt knife-type heater. The periods when the heater was cut in were cumulatively measured by a self-starting electric clock, which was introduced in parallel with the heater. When the heater, in maintaining a water temperature of 98°F., remained on for 40 per cent. of the time, the power expenditure was 50 watts and therefore approximated the heat output of a resting adult human. This condition occurred when the robot was fully clothed and the air temperature was approximately 65°F.

The clothing consisted of a shirt of grey-brown felt 0·3 in. thick over which was placed a black crepe jerkin of moderately open weave. In the experiments to evaluate the factor of moisture, the clothing of one of the robots was dipped in the warm water inside it and kneaded until it no longer dripped when placed on the robot. In experiments to evaluate the factor of temperature, cool water was substituted in one of the robots, and the felt undershirt was omitted from the clothing. When colours were compared, pieces of coloured cloth measuring 2 ft. by 4 ft. were tied around the robots, over the felt shirt.

The coloured cloths employed were satins obtained from Canadian Celanese, Drummondville, Quebec. The luminescent cloths were fluorescent satins produced by the Guarantee Silk and Dye Company, St. Catharines, Ontario. The broadcloths, crepes and chambrays were purchased from post stores at Goose Bay, Labrador.

When the effects of CO_2 and other vapours were to be assessed, an artificial head was placed on the robot. It consisted of a water closet ball-float, wrapped with felt and crepe so that its diameter was 4·5 ins. One ⅜-in. hole served as the mouth, and the vapours were led in through a similar hole in the opposite side. Air and CO_2 were conducted from pressure cylinders with reducing valves, and the flow rate was measured by a rotameter (" flowrator ", Fisher and Porter, Hatboro, Pa.). Vapours of narcotics were generated by bubbling air through the appropriate liquids. A black crepe cape covered the neck and shoulders of the robot.

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Methods.

The robots were set up in wooded glades, and connected (by 200 ft. of cable) to a source of 110-volt 60-cycle A.C. power. They were placed 6 ft. apart, in such a situation as to be shaded as much as possible from direct sunlight, and the surrounding bush was cleared for a radius of 20 ft.

The robots were kept continuously warm by electricity, and were protected against rain (between experiments) by covers of flexible plastic sheeting. The experiments were usually performed in the evening, although mosquitos were often sufficiently abundant for work in the morning or afternoon.

Counts were made of the landing rate of mosquitos on the half-surface of the robot that was visible to an observer, who used a mechanical hand tally. Counts were made simultaneously on each robot and were continued for either 1 min. or 2 min., depending on the abundance of mosquitos. Each time a mosquito alighted on the crepe surface and folded its wings was counted as a landing. The observers stood at a distance of 20 ft. from the robots. It was noticed that there was very little interchange between the swarm of mosquitos around the robots and the swarm around the observers, who never used repellents.

In each experiment, a count was taken on one side, then two successive counts on the other, then one count on the first side ; the observers then interchanged their positions, and the process was repeated. These individual counts were recorded, thus permitting an analysis of variance to be made when the statistical significance of the differences between the totals required evaluation. The values shown in Table I demonstrate that the two robots when in an identical condition attracted similar numbers of mosquitos ; in other words, the " position error " was sufficiently slight not to be a factor.

TABLE I.

Numbers of mosquitos coming in 2-min. periods to each of two robots maintained at 98°F., at Goose Bay, Labrador, 7.30–8.10 p.m., 17th June, 1950. Air temperature, 45–46°F. R.H., 95 per cent.

South man	North man	South man	North man
8	4	15	19
17	6	7	13
7	12	11	6
12	5	25	29
11	9	25	21
10	4	6	10
8	10	6	7
16	23	14	20
Total 89	73	109	125
Student's $t_7=0{\cdot}9$; $p=0{\cdot}40$		Student's $t_7=1{\cdot}3$; $p=0{\cdot}25$	
Grand total	... ...	... 198	198

Biological Material.

The first site was a deep wooded valley with a small stream (Cattawampus Creek) flowing through it, at Camp Borden, near Barrie, Ontario (Pl. XX, fig. 1). The tree cover was second-growth poplar, birch and maple, with some hemlock. During the period of investigation, namely, 23rd to 30th May, the species of mosquitos concerned were almost entirely *Aëdes intrudens* Dyar, *A. trichurus* Dyar, and *A. stimulans* Wlk.*

The second site was a deep wooded valley with a larger stream (" U.S. Pumphouse Creek "), at Goose Bay, Labrador (Pl. XX, fig. 2). The tree cover was composed of balsam fir and black spruce of unusual density and size for that region. During the period of investigation, from 11th June to 31st July, the species of mosquitos involved were mainly *Aëdes punctor* Kby, *A. pionips* Dyar and *A. communis* (DeG.). The population density was high throughout the period, and suitable counts could be obtained even when the temperature was as low as 45°F. The landing rate rose towards evening, the numbers observed at 7–8 p.m. being up to five times as great as the count at 2–3 p.m.

Results.

Effect of Moisture.

A robot whose clothing had been moistened in its own water and left to equilibrate with the air for 30 min., was compared with one whose clothing was dry. At air temperatures between 62° and 65°F., the moistened robot was two to four times as attractive as the dry one for both the groups of species studied (Table II).

TABLE II.

Numbers of mosquitos attracted to moist and to dry clothing at various air temperatures and humidities.

Temperature	R.H.	Species	Moist	Dry
	Per cent.			
65°F.	75	*A. intrudens* etc. .	167	37
65°F.	50	*A. punctor* etc.	175	79
62°F.	65	,, ,,	371	83
60–61°F.	98	,, ,,	159	202
55–56°F.	70	,, ,,	231	481
50°F.	85	,, ,,	206	608
47–50°F.	60	,, ,,	283	442

However, at air temperatures of 55–47°F., the moistened robot was only one-half to one-third as attractive as the dry one. This occurred despite the fact, determined by thermocouples, that the surface of the moistened clothing was 1–3°F. warmer than that of the dry clothing.

Surface moisture was apparently attractive to mosquitos provided the air temperature exceeded 60°F., whereas the situation was reversed at lower temperatures. It may be mentioned that the fourth experiment listed in Table II consisted of two successive determinations, the first being made at an air temperature of 61°F., when the moist man attracted 87 mosquitos as against 58 for the dry one, whereas the second was made half an hour later at temperature of 60°F., when the dry man attracted 144 as against 72 for the moist one.

Effect of Warmth.

A robot that had been filled with cool water was compared with one maintained at 98°F. For these experiments the crepe jerkin alone was used as clothing. The surface temperature of the jerkin enclosing the warm robot proved to be 90°F. at an air temperature of 74°F. without wind, and 81°F. at an air temperature of 58°F. The surface temperature of the jerkin on the cool man was intermediate between the water temperature and the air temperature, at 56°F. when the former was 48°F. and the latter 74°F.

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*Identified by Dr. C. R. Twinn, Head, Household and Medical Entomology Unit, Department of Agriculture, Ottawa, Canada.

TABLE III.

Numbers of mosquitos attracted to a cool robot and to a warm robot.

Air temp.	Temp. of cool man	Species	Warm	Cool
Clothed in jerkin only				
57–59°	62–63°	*A. intrudens* etc.	183	33
66°	64°	*A. punctor* etc.	402	121
67°	64°	,, ,,	265	45
74°	48°	,, ,,	434	71
Clothed in jerkin and felt shirt				
72°	wet, 75°	*A. intrudens* etc.	705	429
65°	dry, 77°	,, ,,	184	174

Table III shows that the warm robot attracted over three times as many mosquitos as the cool one. When the jerkin was the only clothing, the difference in surface temperature of the two robots was between 14 and 17°F.

If both robots were fully clothed, with the felt shirt under the jerkin, the difference in surface temperature fell to only 4°F., and there was no significant difference in the numbers of mosquitos attracted to each. However, if the clothing was wet, that of the warm man was appreciably more attractive than the cool one. This may be due to the ability of the moisture to conduct the body heat out to the surface, as indicated by the higher surface temperature when the clothing was moist.

Effect of Carbon Dioxide.

The clothing of one robot was steeped in water saturated by bubbling CO_2 gas through it, and was compared with the clothing of the other robot, which had been steeped in water only. There was no significant difference between the numbers of mosquitos attracted. This experiment was performed three times with *A. punctor* and other northern species, and once with *A. intrudens* and other southern species. The numbers were respectively (the numbers for the CO_2 treatment first) : 466 vs. 414 ; 162 vs. 162 ; 56 vs. 73 ; 205 vs. 283. The emission of CO_2 vapour from the head of the robot, however, proved to be significantly attractive to mosquitos of both the groups of species studied (Table IV).

TABLE IV.

Numbers of mosquitos attracted to the body when CO_2 was emitted from the head of one of the two robots.

Vapour	Air temp.	Species	CO_2 emitted	No CO_2 emitted
10 per cent. CO_2*	74–76°F.	*A. intrudens* etc.	548	335
10 per cent. CO_2	69–73°F.	*A. punctor* etc.	423 (138)†	323 (86)
100 per cent. CO_2	70°F.	,, ,,	353 (175)	189 (47)
100 per cent. CO_2	68°F.	,, ,,	255 (136)	125 (48)
100 per cent. CO_2‡	55°F.	,, ,,	386 (249)	234 (112)

*Clothing moistened ; in the other experiments the clothing was dry.
†Figures in parentheses denote the numbers of mosquitos coming to the head and shoulders.
‡Compared with a stream of air alone ; others compared with no air-stream.

When a mixture of 10 per cent. CO_2 and 90 per cent. air was emitted at 2 litres per minute, corresponding to the normal human exhalation rate of 200 cc. of CO_2 per minute, 30 to 60 per cent. more mosquitos were attracted to the body of the robot than to the other robot, over which nothing was emitted. When the counts obtained for 10 per cent. CO_2 with *A. intrudens* etc., were analysed for variance to assess the significance of differences, using the t test of Student*, it was found that $t_{15}=5{\cdot}1$ and $p=0{\cdot}0005$; the difference is therefore highly significant. The values obtained with 10 per cent. CO_2 with *A. punctor* etc., were analysed to yield $t_{15}=2{\cdot}7$ and $p=0{\cdot}015$, indicating a moderately significant difference.

If 100 per cent. CO_2 was substituted for 10 per cent. CO_2, and emitted at 2 litres per minute, twice as many mosquitos were attracted to the body as when nothing was emitted. If 100 per cent. CO_2 was compared with air emitted from the other robot at the same rate, it proved to be 60 per cent. more attractive. The differential in the landing rate on the head was somewhat more pronounced than the differential for the body.

Effect of Other Vapours.

Since it had been observed that ground-crew engaged in refuelling aircraft in the north were exceptionally pestered by mosquitos, and since carbon dioxide may act as a narcotic to insects, the attractiveness of certain narcotic vapours such as gasoline were tested (Table V).

TABLE V.

Numbers of mosquitos attracted to robots whose heads emitted air-streams, to one of which certain vapours were added.

Air Temperature	Vapour added to air-stream	Air-stream alone
52°F.	No air-stream : 181 (75)	199 (90)
59°F.	Chloroform : 43 (11)*	72 (45)
58°F.	Gasoline : 79(23)	46 (34)
56°F.	Ether : 214 (101)	156 (79)

*Figures in parentheses denote numbers coming to the head and shoulders.

Gasoline vapour did increase the number of mosquitos landing on the body, but decreased the number landing on the head and shoulders. Diethyl ether (anaesthetic grade) also was significantly more attractive than air alone. On the other hand, the vapour of chloroform was evidently repellent.

The attractiveness of sweat vapours was tested by using one of the crepe jerkins to absorb liquid sweat from the bodies of two men, placing it on a felt-clothed robot, and comparing it with a jerkin of approximately equal moisture content. A total of 172 mosquitos were attracted to the sweaty jerkin as against 97 to the other. Individual counts varied so widely, however, that the Student's t for 15 degrees of freedom was only 2·3, indicating a p of 0·04, significant at the 5 per cent. level.

Effect of Clothing Brightness.

A white broadcloth jerkin was very much less attractive than a black crepe jerkin (Table VI). This was the case even at the lowest light intensities, when the mosquitos were scarcely visible enough to be counted.† The intensity of light reflected from the white broadcloth, as measured by an exposure meter (Weston model 852), was four times that reflected from the black crepe.

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*Derived from the equation :

$$t_{n-1}=\frac{d}{\sqrt{\dfrac{\Sigma d^2-1/n\ (\Sigma d)}{n(n-1)}}}$$

Where d is the numerical difference between the two members of the paired counts (taking sign into account), n is the number of paired counts, and n-1 represents the degrees of freedom.

†On the evening of 7th July, 1950, adult black flies (*Simulium* spp.) were so abundant on the black crepe (landing rate, 300 per min.) that the mosquitos were kept away.

TABLE VI.

Numbers of mosquitos attracted to light and to dark colours.

Colours compared	Light colour	Dark colour	Light value*
Pink and red	27	144	8
	19	102	7
Light-blue and dark-blue	53	60	10
	28	101	8
White and light-blue	15	47	13
	16	30	15
White and black	17	128	1
	14	146	4
	73	274	6
	49	204	17

*The general light conditions prevailing, as indicated by the reading on the exposure meter when directed away from the sun and towards the experimental site.

When a light-blue satin was compared with a dark-blue satin, a pink satin with a red one, and a white satin with a light-blue one, it was found in every case that the lighter cloth attracted fewer mosquitos than the darker one (Table VI). The difference in reflectivity was considerable in the case of the blue cloths, but was slight in the other instances.

TABLE VII.

Numbers of mosquitos attracted to luminescent cloths and to their normal colour matches.

Colour	Luminescent	Normal	Colour	Luminescent	Normal
Light-green ...	13	95	Red	56	148
	22	62		30	51
Dark-green ...	31	161	Purple	11	20
	3	19		26	76
Light-blue ...	11	24	White	37	42
	34	47		51	157

Luminescent satins were much less attractive to mosquitos than their colour matches in ordinary satin (Table VII). All the differences were highly significant ; for example, the counts obtained for the red shade yielded a t_{15} of 3·16 and a p value of 0·01. The luminescent colours showed a greater reflectivity than the normal satins to the extent of 1–2 units on the exposure meter.

All the experiments comparing cloths of different colours, luminosities and textures were performed in duplicate, the first test being made with brand-new cloth, the second after a thorough laundering.

The Effect of Clothing Colour.

Satin cloths were obtained in several deep rich colours and were compared for their attractiveness. Green was considerably less attractive than red. There was no significant difference in attractiveness between red satin and blue satin, or between blue crepe and black crepe (Table VIII). Tests with the exposure meter showed that the green was slightly less reflective than the blue, which was considerably less reflective than the red.

TABLE VIII.

Numbers of mosquitos attracted to various colours compared in pairs.

Dark colours	First colour	Second colour	Light value
Red and blue	156	40	10
	51	53	3
	54	35	17
Red and green	136	42	10
	65	36	9
Blue and black	150	174	8
	160	111	8
Light Colours			
Red and blue	22	70	14
	34	40	11
Red and green	64	22	16
	43	38	9
Blue and green	17	36	11
	46	36	7

When light colours were compared, green was significantly less attractive than red or blue, and red (pink) significantly less attractive than light blue. The reflectivity of the three colours was approximately similar.

Effect of Clothing Texture.

A comparison of black satin with black crepe, and of blue satin with blue crepe, showed that the glossy satin was less attractive than the matt crepe (Table IX). Similarly, red satin proved less attractive than red chambray (broadcloth). The significance of the difference between black satin and black crepe, for example, is represented by $t_{23}=2{\cdot}95$ and $p=0{\cdot}01$, and the other differences were even more significant. In each case the satin showed the greater reflectivity to the extent of approximately 2 units on the exposure meter.

TABLE IX.

Numbers of mosquitos attracted to cloths of various textures.

Black satin	Black crepe	Red satin	Red chambray
175	218	19	37
38	86	21	71
Blue satin	Blue crepe	Khaki nylon	Khaki drill
98	341	23	114
38	121	30	135

A suit of woven nylon,* greenish-khaki in colour, was compared with the ordinary khaki summer service clothing. The top-piece, or parka, was compared with a cotton shirt, and the pants with a pair of khaki drill trousers. The nylon suit was very much less attractive to mosquitos than the usual cotton-drill outfit (Table IX), despite the fact that the reflectivity of the nylon was about one-third of that of the drill garments.

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*Anti-insect suit, 1950 model, developed by the Environmental Protection Section of the Defence Research Board of Canada.

Summary.

1. The responses of adult mosquitos, consisting of several Canadian species of *Aëdes*, were investigated under field conditions by employing heated and clothed robots.

2. Moisture increased the attractiveness of a warm body two to four times when the air temperature exceeded 60°F. ; at cooler temperatures, surface moisture decreased the attractiveness.

3. Warmth increased the attractiveness of a body, so that a robot at 98°F. attracted three times as many mosquitos as one at 50–65°F.

4. It may therefore be concluded that moisture is the major attractant factor when the air temperature exceeds 60°F., and warmth when the temperature is less than 60°F.

5. Carbon dioxide vapour emitted from an artificial head almost doubles the attractiveness of a warm body.

6. The vapours of ether and gasoline were significantly attractive. A sweat-soaked jerkin was more attractive than a water-soaked one.

7. Light colours were less attractive than dark ones, and luminescent dyes decreased the attractiveness of cloths. Green is less attractive than red or blue.

8. Glossy satins were less attractive than matt broadcloths. Greenish-khaki nylon cloth was much less attractive than khaki drill or cotton.

9. There was no evidence of interspecific differences in the responses of the mosquitos investigated.

Acknowledgements.

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Grateful acknowledgement is made of the generous assistance given by Dr. A. C. Burton, Professor of Biophysics, University of Western Ontario, in the design of the apparatus.

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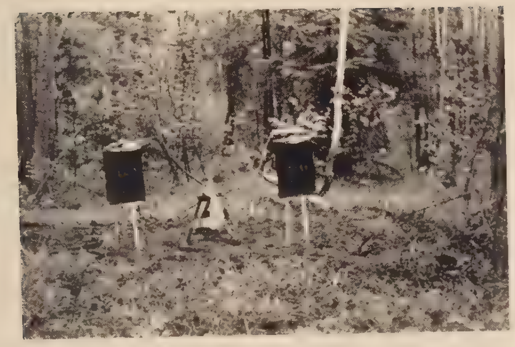

FIG. 1. Robots set up at Barrie, Ontario.

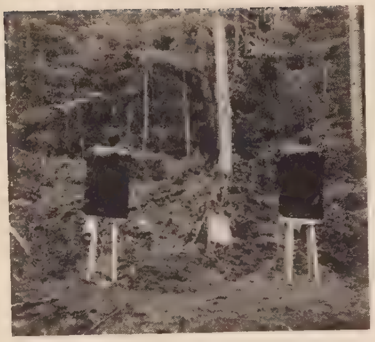

FIG. 2. Robots set up at Goose Bay, Labrador. (Note the model heads).

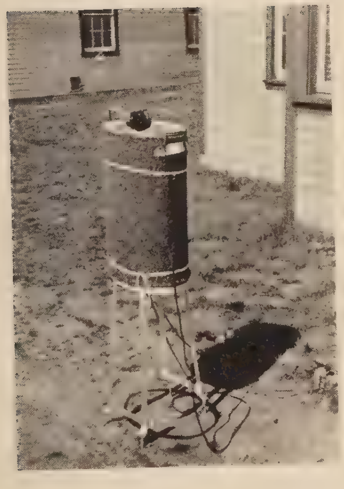

FIG. 3. Detail of assembly of a robot.

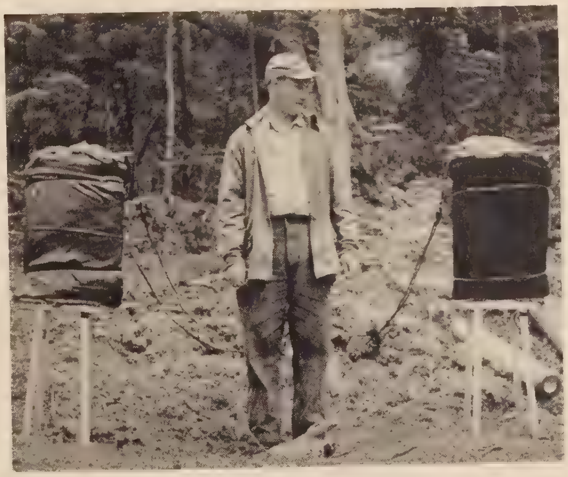

FIG. 4. Size of robot as compared with a 180 lb. man.

DEATH OF TURKEYS FROM ATTACK BY *SIMULIUM GRISEICOLLE* BECKER IN THE NORTHERN SUDAN.

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VP.

Simuliid flies are a well known feature of riverain land in the northern Sudan during months of low Nile. They have been discussed in detail by Lewis (1948). Two species occur, *Simulium damnosum* Theobald, and *S. griseicolle* Becker. The former is larger and darker in colour. The latter is smaller with characteristic grey shoulders and is usually much more numerous. *S. damnosum* bites humans readily, especially round the ankles. *S. griseicolle* is usually less effective in its attempts to bite humans but susceptible persons may be badly bitten. It commonly attacks the head and shoulders and may cause great irritation by invading the eyes, ears, nose and mouth. Temporary protection for up to four hours can be obtained by rubbing the skin with di-methyl phthalate which acts as a repellent against both species.

Birds appear to be the preferred hosts of *S. griseicolle* (King, 1923 ; Lewis, 1948) which by repute is said to be capable of causing the death of poultry, but no substantiated case of this in the Sudan appears to be on record. In North America there are well established cases of deaths among poultry from attack by Simuliids which are sometimes known as "turkey gnats" in that continent. Walker (1927) in Canada reported the deaths of goslings from attack by *Simulium bracteatum* Coq., and Gibson (1930), also in Canada, stated that *Simulium* spp. caused losses among chickens and turkeys. Biester & Schwarte (1948) refer to heavy losses among chickens in western Iowa associated with attacks by swarms of Simuliids which resulted in severe anaemia with haemorrhages at each skin area punctured.

On 2nd December 1949, two hen turkeys each of about five and a half pounds live weight were bought in Khartoum at 11 a.m. They were alive, vigorous and apparently in good health. On superficial examination they appeared to be free from lice and skin wounds. At midday they were brought to the School of Agriculture at Shambat (three miles north of Khartoum on the east bank of the main Nile) where they were tethered to small bushes in the shade of large banyan trees (*Ficus benghalensis*) near a house. They were given whole maize and water and, after eating a moderate meal, they perched in the bushes. At 2 p.m. it was noticed that both birds were surrounded by a cloud of *S. griseicolle*. They did not seem to be distressed but sat passively, making occasional pecks at the flies. At 7.30 p.m. both turkeys were found dead beneath the bushes. The corpses were locked in the boot of a car and next morning, 3rd December, were examined superficially at 7 a.m. No large wounds or bites could be found, but it was noticed that the feathers were full of live *S. griseicolle* which appeared to be biting the dead birds. Round the base of every feather examined were small superficial wounds from which blood had oozed. The birds were sent, unplucked, at 10 a.m. to the Khartoum Veterinary School for a post mortem examination.

On arrival at the Veterinary School, the two birds were examined externally for the presence of lice or mites, wounds or swellings. No vermin could be found nor any lesion to suggest snake or scorpion attack. There was, however, evidence of considerable haemorrhage from the skin as shown by encrusted clotted blood at the base of many feathers all over the body. No Simuliids were found among the feathers at this examination.

The carcasses were then plucked and prepared for post mortem examination. Externally the skin showed innumerable small punctured wounds all over the body, no part of which had been left untouched : head, neck, back, wings, thorax, abdomen and thighs all showed tiny punctured wounds which had bled profusely, the blood clotting and encrusting round the punctures. In many places, as for instance round the vent, the lower abdomen, and the sides of the thorax, the wounds were so numerous as to coalesce to form extensive areas of haemorrhage, each of about 25 square centimetres. In these areas, it was impossible to find any normal skin. The appearance suggested severe bruising, but deflection of the skin showed that the injuries were entirely superficial and did not extend to the subcutaneous or muscular tissues.

Examination of the internal organs of both birds showed no abnormality except the complete absence of blood. The appearance of each carcass was that of a bird efficiently killed and bled for the table ; pale musculature, pale organs slightly reduced in volume, no prominence of circulatory vessels and no blood in the cavities of the heart. The extent of the anaemia can be gauged when it is stated that there was not sufficient blood in any of the heart cavities with which to make a blood film for examination.

Bacteriological examination was carried out and proved negative, and as a precaution, a chemical examination of the crops and gizzards of both birds was made, although there was nothing in the contents or appearance of these organs to suggest poisoning. This examination gave negative results for common mineral poisons including arsenic and phosphorus. Apart from evidence of the last meal fed to the turkeys, the crops of each contained a few Simuliids showing that the birds had been making desultory efforts to remove the flies. Biological examination was not possible, but again nothing was observed to suggest that the deaths had been associated with virus infection.

All the evidence pointed to the conclusion that the deaths of the birds had been caused by severe and rapid anaemia following extensive external haemorrhage, presumably due to massive attacks by biting flies.

S. griseicolle is always present at Shambat to a greater or lesser extent during the months of low Nile from November to June. Experienced residents say, however, that its incidence during the winter of 1949–1950 was much greater than usual. Poultry at Shambat are commonly protected by smoke from smudge fires, but the turkeys in question were not so protected and their death in a matter of hours indicates the intensity of the attack. *S. griseicolle* therefore may well be responsible for some of the deaths usually attributed to snakes or scorpions, and these small flies must be regarded as a factor of some importance in poultry production on riverain land in the northern Sudan.

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THE EFFECT OF RELATIVE HUMIDITY ON THE ACTIVITY OF THE TROPICAL RAT FLEA *XENOPSYLLA CHEOPIS* (ROTHS.) (SIPHONAPTERA).

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(Plate XXI.)

The adaptive significance of aggregation on the dry side of an alternative chamber for certain insects such as *Locusta migratoria* (L.) (Kennedy, 1937), *Tenebrio molitor* L. (Pielou & Gunn, 1940) and *Culex fatigans* Wied. (Thomson, 1938) is not known. The present work shows that this reaction occurs in *Xenopsylla cheopis* (Roths.) and a series of experiments to discover its survival value for this flea is here described.

Material and Methods.

The fleas were maintained by Leeson's (1932a) technique. Except where stated to the contrary all fleas used in experiments were unfed, of mixed sexes and ages and each result was the mean for four experiments. The technique for obtaining fleas up to one day old is given by Leeson (1932b). The sexes were separated by examining single fleas in a narrow glass tube under a binocular microscope.

Flat chambers were used to observe the activity of ten fleas or their reaction to alternative humidities (Gunn & Kennedy, 1936) ; each chamber consisted of two petri dishes 9 cm. in diameter fitted edge to edge. The upper dish had a hole in the top and a bolting silk platform stuck to the rim with balsam. A stout elastic band bound the top dish to the bottom one to make the apparatus air-tight. In order to study activity at a particular relative humidity, 40 cc. of a solution of glycerine and water of the required dilution (Johnson, 1940) were put into the bottom dish. An alternative humidity chamber was made by fixing a glass partition across the centre of the bottom dish dividing it into two. Two different solutions could then be used. The chambers were placed in a large incubator at 25°C., insulated with cotton wool against vibration, and the humidity in the chambers measured within 5 per cent. R.H. by paper hygrometers (Mellanby, 1933). A mirror inside the incubator made it possible to inspect all parts of the apparatus through a window which was made light proof when not in use.

Activity at a particular humidity was studied by putting five fleas into the upper part of the chamber, sealing the hole with transparent tape, and then recording the number of fleas jumping and crawling at successive minutes for ten minutes. The dispersal of fleas in atmospheres of graded humidities was studied by putting ten fleas into an alternative chamber and recording the number in the side of higher humidity at five-minute intervals for an hour. The average number of fleas on the dry side expressed the degree of aggregation. The control was a chamber at uniform humidity. With the exception of an experiment on light response the incubator was illuminated only to take readings.

Two statistical methods were employed, the *Chi squared* (χ^2) test to assess the homogeneity of repeated experiments, and the *t* test by means of which the significance of the difference in results between different experiments was evaluated.

There was a wide variation in results in repeated alternative chamber experiments, the extent of which is shown in the tables, which give the values of the Probability P. When P=1 the probability of any difference occurring by chance is 100 per cent. The interpretation of P for the *t* test is the same, so that when P=·001 the probability of the difference in results occurring by chance is 0·1 per cent. and when P=·80 the probability of the difference being a chance one is 80 per cent. It is conventional to adopt a 5 per cent. level of significance. This means that when P=·05 or less the difference between results is statistically significant.

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Experiments with Alternative and Activity Chambers.

Whenever *Xenopsylla* reacted to alternative humidities it collected in the drier side of the apparatus. Furthermore, in several alternatives in which the higher humidity was the same and the lower humidity different, the number of fleas on the dry side increased as the gradient increased (Table I).

TABLE I.

Alternative humidities	Gradient per cent.	Number on dry side	Control	P for χ^2		P for t	
				Alternative chamber	Control	Alternative chamber	Control
85–92·5	7·5	6·62	5·06	·001	·20	·05	·80
79–90·5	11·5	8·1	4·98	·001	·02	·1	·99
70–89·0	19·0	9·1	4·98	·001	·05		

When the gradient (*i.e.*, the percentage difference between the two sides) was kept constant, the number of fleas on the dry side was greater if the alternatives were towards the moister end of the humidity scale (Table II).

TABLE II.

Alternative humidities	Gradient per cent.	Number on dry side	Control	P for χ^2		P for t	
				Alternative chamber	Control	Alternative chamber	Control
21–35	14	5·02	5·13	·02	·001	·70	·99
39–51	12	5·35	5·06	·10	·001	·10	·80
54–72	18	7·19	4·82	·02	·02	·30	·70
79–93	15	7·33	4·79	·30	·70		

While the trend of the results was distinct (Tables I and II) there was, with one exception, no statistically significant difference between successive experiments.

Two aspects of locomotion were included in the term " activity ". Firstly, the percentages of fleas seen to move at one observation : this was referred to as " locomotor activity ", and secondly, the speed of locomotion. The results for one repetition of a typical experiment on activity are given in Table III. In this experiment the relative humidity was 100 per cent. The percentage of fleas seen

TABLE III.

Successive minutes	1	2	3	4	5	6	7	8	9	10	Total	Per cent.
Number jumping ...	3	5	2	1	0	2	2	0	0	0	15	30
Number crawling...	2	0	1	0	0	0	0	0	0	0	3	6
Number moving ...	5	5	3	1	0	2	2	0	0	0	18	36

to be jumping or crawling at any one observation was found as follows. As there were ten observations on five fleas the total figure possible for each category of movement was 50. The sum of fleas jumping and crawling is the actual number observed out of a possible 50. Therefore when this figure is doubled one obtains a percentage. This was the average number of fleas expressed as a percentage, which were active in a group of ten observations during ten minutes. The results for experiments at different relative humidities are given in Table IV. There was evidently a sharp increase in the percentage of moving fleas above 85 per cent. relative humidity. It was obvious from inspection of moving fleas that they move faster by jumping than by crawling. At high humidities a great proportion of the total movement was by jumping (fig. 1). The disturbance of the fleas striking the

TABLE IV.

Relative humidity	Per cent. jumping	Per cent. crawling	Per cent. movement	P for χ^2	P for t
33	2·0	6·5	8·5	·70	·02
85	6·5	6·5	13·0	·80	·10
93	10·0	7·5	17·5	·50	·02
100	22·0	11·0	33·0	·30	

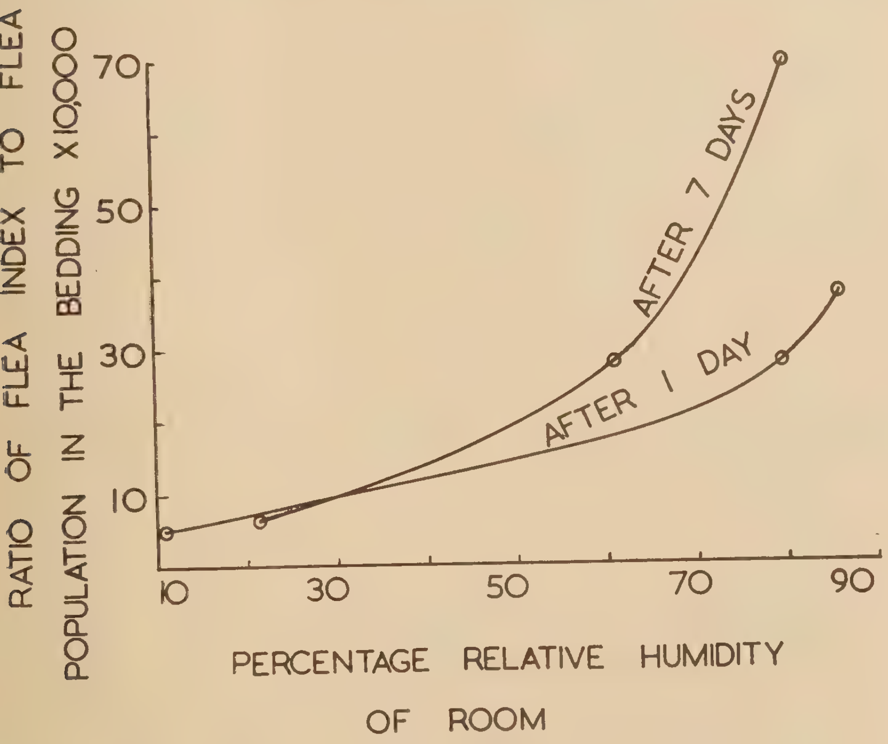

Fig. 1.—Activity of *X. cheopis* in relation to relative humidity.

platform when they were tipped into the activity chamber appeared to be the primary stimulus (Key, 1936) to activity. This was shown by a decline in activity in successive minutes after the fleas were tipped into the chamber. This decline was rapid at low humidities. Table V shows the combined results of four experiments, each with five fleas, for each humidity.

TABLE V.

Relative humidity	Number of fleas moving in successive minutes									
	1	2	3	4	5	6	7	8	9	10
33	10	4	3	0	0	0	0	0	0	0
85	14	9	1	1	1	0	0	0	0	0
93	16	10	5	0	1	2	1	0	0	0
100	16	15	11	4	5	7	5	2	1	0

In a chamber at one humidity *X. cheopis* showed little activity after a few minutes, but when exposed to alternative humidities they moved about for some time. One concluded that the gradient of humidity stimulated the fleas to activity in addition to the initial disturbance.

Some unfed fleas, up to one day old, were kept at 70 and others at 90 per cent. relative humidity for 24 hours, and their reaction to the alternatives 65 and 85 per cent. R.H. studied. Previous treatment had no significant effect with the exception that after exposure to 70 per cent. R.H. males collected on the dry side more strongly than the females, P=·001 (Table VI). Unfed males, up to one day old, kept at 90 per cent. relative humidity for five days aggregated intensely in the drier of the alternatives 65 and 85 per cent. R.H. Similar fleas kept at 70 per cent. R.H. reacted weakly to the same alternatives (Table VII).

TABLE VI.

Sex of fleas	Previous R.H.	Number on dry side, alternative chamber	Control	P for χ^2		P for t	
				Alternative chamber	Control	Alternative chamber	Control
Female	70	6·83	5·81	·001	·30	·70	·02
	90	8·23	5·66	·20	·10		
Male	70	8·18	4·62	·05	·30	·40	·02
	90	7·93	5·57	·95	·50		

TABLE VII.

Previous R.H.	Number on dry side, alternative chamber	Control	P for χ^2		P for t	
			Alternative chamber	Control	Alternative chamber	Control
70	6·33	5·39	·05	·80	·001	·80
90	9·31	5·33	·50	·50		

Fleas were then kept at 70 per cent. or 90 per cent. R.H. for five days and the activity of each group in atmospheres of 60 and 90 per cent. R.H. was studied. Table VIII shows that the fleas were more active after exposure to 90 per cent. R.H. than after exposure to 70 per cent. R.H.

TABLE VIII.

Activity after 5 days exposure to	R.H.	Per cent. jumping	Per cent. crawling	Per cent. movement	P for χ^2	P for t
70 per cent. R.H.	60	1·5	3·5	5·0	·20	·05
	90	11·5	8·0	19·5	·50	
90 per cent. R.H.	60	7·5	6·0	13·5	·90	·05
	90	21·0	8·5	29·5	·30	

It was concluded that there was a definite relationship between activity and aggregation in the alternative chamber. Activity varied directly (although not in a simple linear manner) with relative humidity. The degree of aggregation depended upon the difference in activity which occurred in fleas at different humidities; thus the greater activity in a wet atmosphere led to aggregation in the dry side in the alternative chamber. The degree of aggregation is affected not only by the percentage difference of the alternative humidities, but also by their position in the humidity scale.

Blatta (Gunn, 1940) and *Ptinus* (Bentley, Gunn & Ewer, 1941) show a diurnal rhythm of activity. During a normal day's work no such rhythm of activity was found in unfed female fleas up to one day. Nor was indication found of any sex difference in activity in fleas taken directly from debris jars (Leeson, 1932).

Some unfed female fleas up to one day old were put in a jar containing a mouse and after 24 hours those which had fed were selected. The intensity of aggregation of these fed fleas was compared with that of unfed females of the same age to the

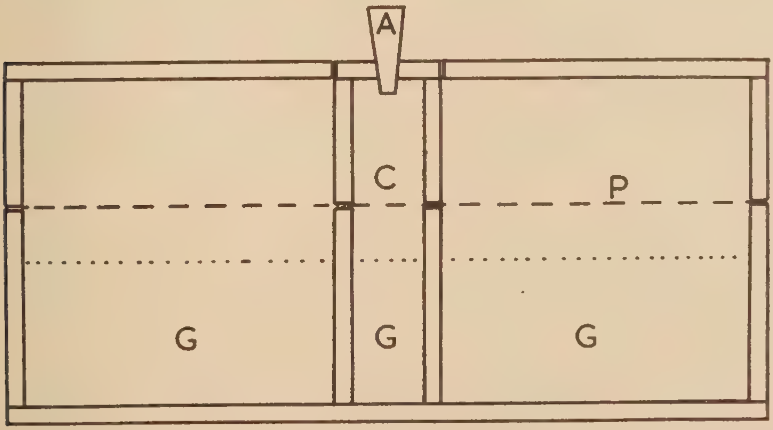

Fig. 2.—(A) Cork knob for lifting up central lid, and which fills hole through which flea is introduced into central compartment C. (P) Platform of bolting silk. (G) Glycerine-water solutions.

alternatives 80 and 90 per cent. R.H., and no significant difference in degree of reaction was found. Degrees of aggregation were 8·4 for unfed and 7·8 for fed fleas, P=·1.

Nosopsyllus fasciatus (Bosc) reacted in the same way as *X. cheopis* to uniform and alternative humidities. The degree of aggregation to the alternatives 80 and 95 per cent. was 7·1. At 95 per cent. relative humidity 10 per cent. of the fleas were recorded as jumping and 8·5 per cent. crawling while 3·5 per cent. were jumping and 13·5 per cent. crawling at 80 per cent. relative humidity.

The effect was studied of preliminary short exposures to different humidities on the reaction of *X. cheopis* to alternative humidities. A chamber (fig. 2) was designed in which a flea could first be confined in a small central compartment. When the central lid was raised the flea was exposed to the alternatives 50 and 90 per cent. R.H. Records for 40 fleas used in each experiment showed that more *X. cheopis* moved into the dry atmosphere after confinement at 50 per cent. R.H. for five minutes than after confinement at 90 per cent. R.H. for the same time (Table IX). It has already been shown (Table VII) that previous exposure to a low humidity for five days had the opposite effect ; it decreased the degree of aggregation.

TABLE IX.

	Alternative Chambers				Control	
	After confinement at 50 per cent. R.H.		After confinement at 90 per cent. R.H.		After confinement at 90 per cent. R.H.	
Alternative relative humidities	50	90	50	90	90	90
Xenopsylla cheopis ...	36	4	26	14	20	20
Nosopsyllus fasciatus ...	27	13	27	13	19	21

In this paper the use of the term klino-kinesis is to be taken to mean random turning movements where the rate of turning is affected by the intensity of the stimulus. "Adaptation" in conjunction with klino-kinesis enables aggregation to occur more effectively than by klino-kinesis alone. In other words klino-kinesis, coupled with adaptation in the presence of a uniform stimulus, results in the rate of turning falling to a basal level. An explanation of the effect of short confinements might be to assume that a high klino-kinesis occurred in *X. cheopis* (Roth.), and that after five minutes the flea was adapted to the humidity in which it was confined so that its rate of turning was basal. The mechanism may then be explained as follows :—

Confined at 90 *per cent. relative humidity.*

(*a*) As the adapted flea moved towards 50 per cent. R.H. its rate of turning remained unchanged for it was basal.

(*b*) As the flea moved towards 90 per cent. R.H. its rate of turning remained unchanged for it was adapted to that simulus.

Confined at 50 *per cent. relative humidity.*

(*a*) As the flea moved towards 50 per cent. R.H. its rate of turning remained unchanged for it was adapted to that stimulus.

(*b*) As the flea moved towards 90 per cent. R.H. its rate of turning increased. This increased the likelihood of the flea avoiding the high humidity.

There was no direct evidence that a high klino-kinesis occurs in *X. cheopis*. Indeed, this would be most difficult, if not impossible, to demonstrate by Ullyott's (1936) method. When one observed *X. cheopis* moving about it was sometimes seen to turn to one side between intervals of crawling and jumping. However, *Nosopsyllus fasciatus* showed similar turning movements which did not appear to be klino-kinetic, for short confinements, as for *X. cheopis*, had no effect on its reaction to the alternatives 50 and 90 per cent. R.H.

The Effect of Relative Humidity on Host-finding.

A roll of lint, tightly sewn together, was dropped into a jar infested with fleas and the number which jumped on to the roll was counted at three ranges of humidity and a temperature of 27–28°C. The details of the apparatus are shown in fig. 3. A slow stream of air of fairly constant relative humidity was passed through a jar. Glycerine or potassium carbonate and sodium tartrate solutions gave the required

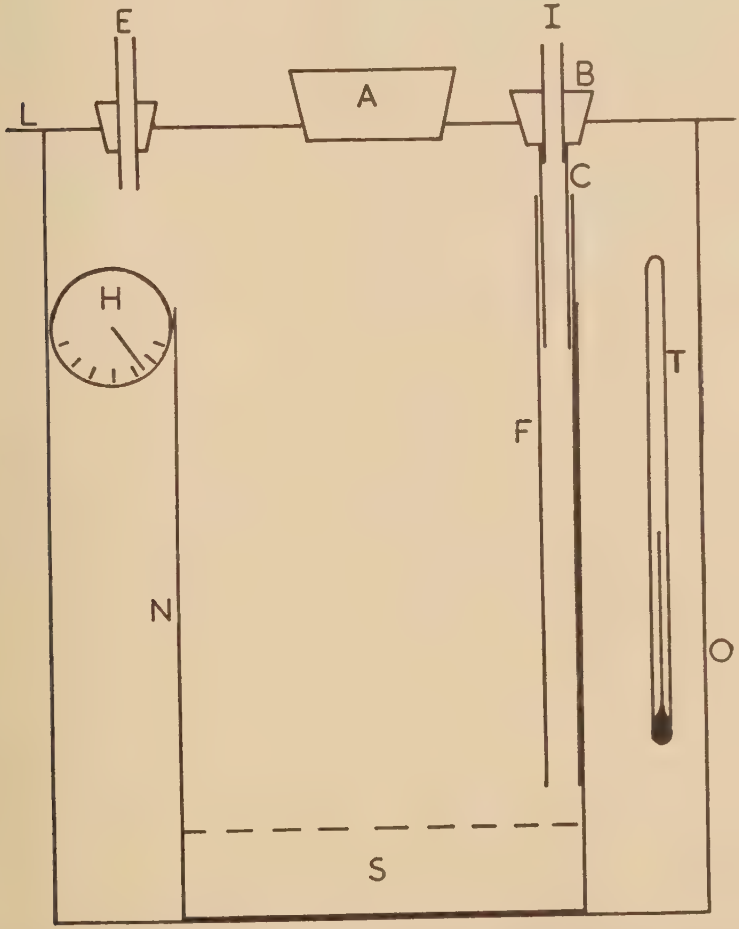

Fig. 3.—(A) Cork to hole A. (I) Air inlet. (E) Air outlet. (L) Lid. (T) Thermometer. (H) Edney hygrometer. (O) Outer jar. (N) Experimental chamber. (S) Sawdust. (F) Glass tube which conveys air to surface of sawdust.

ranges of humidity, and conical flasks immersed in a water bath contained the solutions. From the flasks, air was conveyed by a glass tube (F) to the surface of a thick layer of sawdust in the bottom of the experimental chamber. The apparatus was constructed so that the experimental chamber could be taken out of the outer jar. A short piece of rubber tubing C conveyed air into the glass tube F. When bung B was removed C was withdrawn. The lid could then be taken off the large jar and the experimental chamber taken out with tube F attached.

When the hygrometer showed that the humidity was in the required range 20 fleas were tipped on to the sawdust through hole A. After five minutes the thermometer and hygrometer were read. A roll of lint was then dropped through hole A on to the sawdust. After one minute the roll was removed by tongs on to a large sheet of cotton wool, and the fleas which had jumped on to the roll counted and the sex determined. The sawdust was then tipped into a battery jar whence the remaining fleas were removed with a suction pump and sexed. The apparatus was refitted and the procedure repeated. The number of each sex which jumped on to the roll of lint was known and also of those which remained in the sawdust, and it was possible to calculate for each sex the proportion of fleas which jumped on to the lint roll. Table X shows the results. Each result is the mean of ten repeated experiments. The results show that most fleas jumped on to the roll of lint at the highest humidities, and that relatively more females than males occurred on the roll.

TABLE X.

Range of relative humidity	Average number of fleas on lint roll	P for χ^2	P for t	Proportion which jumped on to roll	
				Males	Females
32–37	·7	·02	·60	·03	·04
72–75	1·1	·01	·01	·07	·8
82–87	4·2	·05		·19	·3

This procedure was repeated with a month-old rat in place of the roll of lint. The rat was taken out of the experimental chamber and lightly anaesthetised with chloroform. The fleas on it were removed, counted and their sex determined. Table XI shows that more fleas jumped on to the rat than on to the lint roll, and that a significantly larger number reached the rat at 70–78 per cent. R.H. than at 32–38 per cent. R.H. It was concluded from this host-finding experiment that the disturbance, which the rat produced, stimulated the fleas to activity and as activity

TABLE XI.

Range of relative humidity	Average number of fleas on rat	P for χ^2	P for t	Proportion which jumped on to rat	
				Males	Females
32–38	7·5	·001	·01	·71	·46
70–78	12·8	·02	·1	1·80	3·08
83–88	10·1	·01		·6	1·22

is greater at higher humidities most fleas reached the host at the high humidities. A high klino-kinesis may also have contributed towards host-finding. The result for the highest range of humidity, in which one of two rats was used alternately, was inconsistent with these conclusions. In each case the rat moved about little at 83–88 per cent. R.H. compared with other ranges of humidity and this perhaps accounts for the low number of fleas which jumped on to the rat at this range. It was thought that the rats became afraid as the experiment continued and fear eventually immobilised them.

An Artificially Infested Rat Colony (Plate XXI).

A colony of 25 buck hooded *Rattus norvegicus* was maintained in a room supplied with a bedding of wood turnings. The bedding stretched round two sides of the room as a belt 3½ ft. wide and 3–4 inches deep. The rats weighed 140–300 gm. and 20 of them were individually marked. A fan and an electric fire were installed which kept the room at about 25°C. The humidity was raised by placing a tray of water over the electric fire and another tray beneath the fan (Pl. XXI, fig. 2). To raise the humidity still further the floor was finely sprayed with water on two occasions. A thermohygrograph was placed 5 ft. above the floor on a north-easterly wall and a Cambridge distance thermometer (Pl. XXI, fig. 1) measured the temperature of the bedding. The humidity of the bedding was measured by a modified Edney hair hygrometer (Pl. XXI, fig. 3).

The bedding was infested with *X. cheopis*, in all stages, by scattering the contents of debris jars (Leeson, 1932a) over it. The rats took food and water from dishes put into a cage away from the bedding. After the room had been infested for a week, marked rats were caught with a cage trap (Pl. XXI, fig. 4) and their fleas removed. Rats were again caught and their fleas removed for the following three days. By this means the flea index, which is the average number of fleas per rat, was obtained, (*a*) for rats exposed one day to infestation, (*b*) for rats exposed seven days to infestation.

This procedure was repeated twice corresponding to two successively higher humidities of the room. The adult flea population was estimated for each range of humidity studied by removing samples of bedding ; these samples were tipped into one end of a large glass tank (Pl. XXI, fig. 2) where a light was placed. The fleas moved towards the other end and were collected in a specimen tube attached to a suction pump. They were counted and their sex identified.

In relation to size of flea population, the number of fleas on the rats increased with a rise in the relative humidity of room and bedding. Furthermore, this relationship was more distinct for those rats which had been exposed for a week to infestation (Table XII ; fig. 4). A number of factors may have contributed towards

TABLE XII.

Rats exposed to infestation for	Room		Bedding		Flea index	Flea population in bedding	Ratio of flea index to flea population × 10,000
	Temp.	R.H.	Temp.	R.H.			
1 day	26·7	12	21·0	28	1·4	2,705	5·1
	24·3	80	20·4	74	2·2	776	27·9
	25·75	87	22·5	83	2·0	541	37·4
7 days	25·0	22	19·36	—	1·8	2,705	6·7
	27·5	62	21·29	—	2·1	776	27·7
	24·25	80	20·93	—	3·8	541	70·0

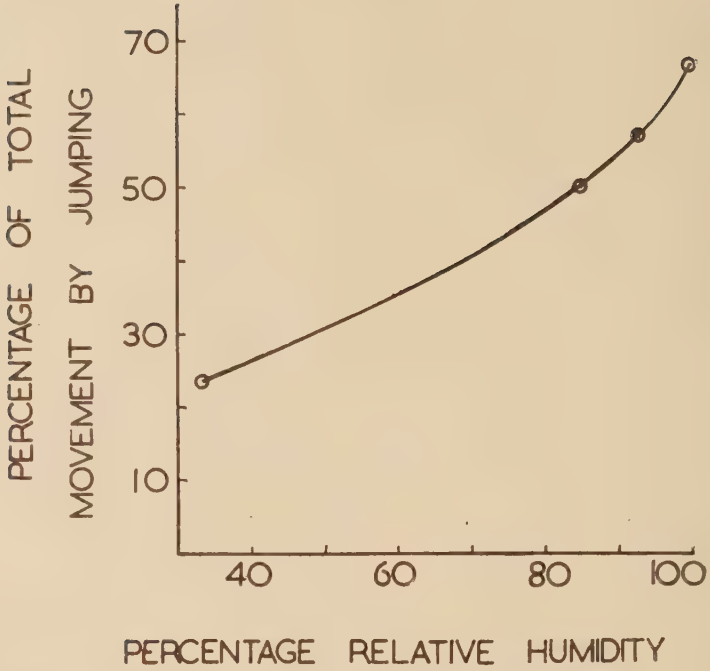

Fig. 4.—Relation between flea index on rats and flea population for different relative humidities and lengths of exposure to infestation.

the fall in adult flea population which occurred during the experiment. Pre-imaginal stages possibly desiccated and died during the first week so that no adults emerged. Some adults may have died because the debris jars contained fleas of mixed ages, and others may have been eaten by the rats. The flea index and the ratio of the flea index to flea population in the bedding for each of the sexes is shown in Table XIII.

TABLE XIII.

Rats exposed to infestation for	Relative humidity of room	Flea index		Ratio of flea index to the flea population in the bedding ×10,000	
		Males	Females	Males	Females
1 day	12	0·9	0·5	5·7	4·3
	80	1·4	0·8	23·7	40·8
	87	1·0	1·0	36·4	38·6
7–10 days	22	0·9	0·9	5·9	7·5
	67	1·9	0·9	30·1	49·5
	82	2·5	1·2	87·4	46·4

On the Position of the Humidity Receptors.

The degree of aggregation of fleas whose maxillary palps were completely or almost completely removed was compared with that of intact fleas.

The maxillary palps were amputated on a sheet of lead on the stage of a binocular microscope. A flea anaesthetised by ether was placed on the sheet and held by fine forceps with the ventral side upwards. The palps were then severed with a fine scalpel. There was a high mortality with this technique. The fleas were exposed to the alternatives 83 and 93 per cent. R.H. The degree of aggregation of operated fleas was 7·9 and intact fleas 8·1. As the difference in degree of aggregation between operated and intact fleas was not significant, P=·70, it was concluded that humidity receptors were not confined to the maxillary palps, if indeed there were any there.

After studying the dispersal of these fleas they were killed and the site of the palps examined. The basal segment was often not removed and the proximal two segments were intact in two fleas.

Discussion.

Arthropods which collect in the dry side of the alternative chamber are not found in dry places in nature. This lack of survival value has been resolved in *Blatta* (Gunn & Cosway, 1938), *Ptinus* (Bentley, 1944) and *Ixodes* (Lees, 1948) for they aggregate in the wet air after desiccation, but the present work has not demonstrated this for *X. cheopis*. Many fleas died which were kept at 70 per cent. R.H. for five days, but those which survived nevertheless aggregated in the dry side.

X. cheopis has a wide geographical distribution (Buxton, 1941) but is most numerous in tropical regions. Its hosts are rodents and principally rats. Even in dry climates there is a high humidity in a rat hole (Buxton, 1932) and presumably a higher humidity in the nest itself. Fleas of the genus *Xenopsylla* are repelled by strong light and are most active in saturated or nearly saturated air. It is possible that they move to and fro in rats' burrows by reactions to humidity and light. Furthermore, when this activity is stimulated mechanically at high humidities it is greatly increased for a few minutes. This burst of activity would contribute towards the fleas reaching a passing host. From the present experiments on host finding it is concluded that the high humidity in a rat's burrow has an important effect as a secondary stimulus (Key, 1936). A rat moving about in a burrow would be expected to disturb fleas and this disturbance to produce a momentary increase in activity in the fleas which is most intense at high humidities. It is also possible that a high klino-kinesis plays a part in rapid host finding at high humidities.

Specific receptors have been located in some arthropods which collect in the dry side of the alternative chamber, *Tenebrio* (Pielou, 1940), *Pediculus* (Wigglesworth, 1941) and *Ixodes* (Lees, 1948). Receptors of this kind may act as little hygrometers (Wigglesworth, 1941). Great sensitivity at high humidities would be accounted for by the receptors taking up more water at high levels than they lose at low ones. This is a general property of hygroscopic substances. *X. cheopis* falls into this class and would be expected to react to relative humidity rather than saturation deficiency. This has also been indicated for *Tenebrio* (Pielou & Gunn, 1940) and *Culex* (Thomson, 1938).

Hirst (1927) found that unfed congregated fleas bit readily but isolated fleas did not feed. He also showed that isolated fleas survived longer than congregated ones and stated that "owing to the increased activity of congregated fleas and the corresponding expenditure of energy such fleas appear to require more frequent nourishment". The results obtained by this worker, and in the rat colony experiment described above, seem to indicate that at high relative humidities fleas require frequent blood meals owing to their high activity. *Pulex irritans* L. lays more eggs when given two blood meals than when given one meal a day (Bacot, 1914). It is possible then that if fecundity increases with number of blood meals it is also greater at high relative humidities. *X. cheopis* feeds copiously and apparently wastefully (Hirst, 1927) for during feeding it defecates what appears to be unchanged blood. Water loss is not concerned, for the adult has considerable powers of water conservation (Leeson, 1932, Wigglesworth, 1932). One biological value of copious feeding is that the faeces of the adult flea supply the larvae with food (Sikes, 1931). It is also possible that the adult flea may require some foodstuff present in the blood in very small quantities.

The average number of fleas per rat, or the flea index, has long been recognised as a measure of flea population. In many cities such as Bombay a rise in the flea index coincides with a high incidence of epidemic bubonic plague cases (Plague Commission Reports, 1908). But the flea index may be greater during the off-season for plague (Webster and Chitre, 1930). Other factors are therefore important in plague transmission. Webster and Chitre (1930) observed that there was a smaller proportion of infected *X. brasiliensis* (Baker) during the plague season. They concluded that in the plague season favourable conditions for "blocking" occur thus allowing a larger proportion of infected fleas to transmit the infection. They found that during the plague season *X. cheopis* leaves a dead rat more quickly than in the off-season. This observation suggests that this flea is highly active during the plague season and that this is a factor in plague transmission. It has been shown (Table XII) that while the *X. cheopis* index is not necessarily larger, the proportion of fleas on the rats is greater at high relative humidities. It is possible that the wandering habits of rats (Hinton, 1931) lead to wide dissemination of fleas under these conditions. One supposes that the infection is rapidly spread from rat to rat at high humidities through infective blood meals and flea faeces (Bacot and Martin, 1914). When many rats die of plague the fleas turn to man.

High saturation deficits have an adverse effect on the pre-imaginal stages of fleas (Petrie & Todd, 1923, Buxton, 1938, Edney, 1947). In nature there is a lag between fluctuation in humidity as measured by relative humidity (Plague Commission Reports, 1910) or saturation deficiency (Petrie & Todd, 1923) and the flea index. This lag is attributed to the effect of the drying power of the air on the development of *X. cheopis* (Petrie & Todd, 1923). It may be that relative humidity affects the fecundity of *X. cheopis* and also contributes towards this lag. Climate can operate in many ways to control the incidence of epidemic bubonic plague: temperatures above 85°F. reduce the power of the flea to infect (Hirst, 1927); high saturation deficiencies have an adverse effect on the pre-imaginal stages; low relative humidities reduce activity and possibly fecundity. But climate is not the sole factor; the composition of nesting materials for example is important (Edney, 1947; Sharif, 1948). Brownlee (1918) has shown that epidemics can occur under widely different conditions of temperature and humidity.

The factors which affect the ratio of male to female *X. cheopis* on a rat are apparently complex. Hirst (1927) found that female *X. cheopis* captured off rats survived longer than males. This is supported by the fact that after repeated blood meals female *X. cheopis* survive longer than similarly fed males (Leeson, 1936). The short life of the male is correlated with its need to feed more frequently than the female (Hirst, 1927). The present rat colony experiments do something to support this. At the highest range of humidity a larger proportion of males than females were on the rats (Table XIII). Webster & Chitre (1930) also found the proportion of male *X. cheopis* on rats to males on the floor of rat cages was greater than for females. But this explanation appears inadequate. At the intermediate ranges studied, 67 and 80 per cent. R.H., there was a greater proportion of females than males on the rats. Buxton (1938) found a higher proportion of female than male *X. cheopis* on mice. The present work shows that while the intensities of aggregation of males and females are the same to the range 80–90 per cent. R.H. the females find their host more quickly than males (Tables X and XI). Hirst (1927) has pointed out two factors which influence the ratio of male to female *X. cheopis* on a rat. Firstly, when a rat dies of plague the fleas leave the cooling body and as the climatic conditions become unfavourable a greater proportion of females to males survive to find a fresh host. Secondly, females emerge before males.

There are similarities between the effect of relative humidity on the behaviour of fleas and its effect on other blood-sucking insects. *Culex pipiens* L. is most active at 94 per cent. R.H. (Rudolphs, 1925). Lewis (1933) found that *Aëdes aegypti* (L.) bites readily at 90 per cent. R.H. but little at low humidities, though this was not confirmed by Lumsden (1947) who used a slightly different apparatus. Unfed *Anopheles gambiae* Giles bites readily at low but not at high humidities (De Meillon, 1937) ; it resembles *X. cheopis* in that relative humidities which produce greatest locomotor activity are optimal for biting activity. But the behaviour of *Culex fatigans* is anomalous. Although locomotor activity is unaffected by humidity (Thomson, 1938) under natural conditions *Culex fatigans* bites man more readily at 50 per cent. R.H. than 40 per cent. R.H. (Gill, 1921).

Summary.

1. The reactions of *Xenopsylla cheopis* Roths. to uniform and alternative relative humidities have been investigated.

2. *X. cheopis* aggregates in the dry side of the alternative chamber. There is an indication that the intensity of aggregation increases with increase in percentage difference between the alternatives and that it is also high when the alternatives are high in the scale.

3. Locomotor activity and speed of movement increase with rise in relative humidity.

4. Males kept at 90 per cent. relative humidity for five days aggregate intensely in the drier of the alternatives 65 and 85 per cent. R.H. Similar fleas kept five days at 70 per cent. relative humidity react weakly.

5. There is greater activity after five days' exposure to 90 per cent. relative humidity than after exposure to 70 per cent. R.H. for the same duration.

6. One concludes that high activity in wet air contributes towards aggregation in the dry side and that the intensity of aggregation is affected by the difference in activity which occurs in the fleas at different relative humidities.

7. The intensity of aggregation to the alternatives 80 and 90 per cent. relative humidity is unaffected by sex, or by the remains of a blood meal.

8. A high klino-kinesis may occur in *X. cheopis*.

9. *Nosopsyllus fasciatus* reacts in the same way as *X. cheopis* to uniform and alternative humidities, but there is no evidence of a high klino-kinesis.

10. When a roll of lint is dropped into a vessel infested with *X. cheopis* the number which jump on to it increases with relative humidity. The number which jumped on to a month-old rat did not show a clear relationship to humidity. Relatively more females than males jumped on to the roll of lint and rat.

11. A colony of 25 buck rats was infested with *X. cheopis*. In relation to size of flea population the flea index increases with rise in relative humidity. The proportion of each sex on a rat, to its respective number in the bedding material, does not appear to bear a simple relationship to humidity.

12. Humidity receptors are not confined to the distal three segments of the maxillary palps if indeed there are any there.

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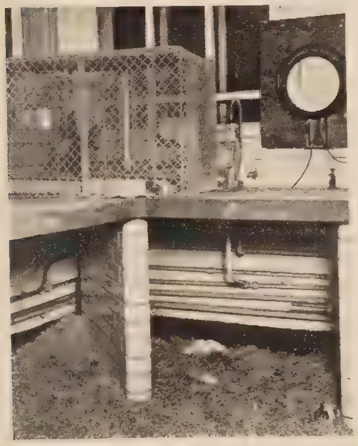

FIG. 1. Distance thermometer. Electric fire placed over tray of water.

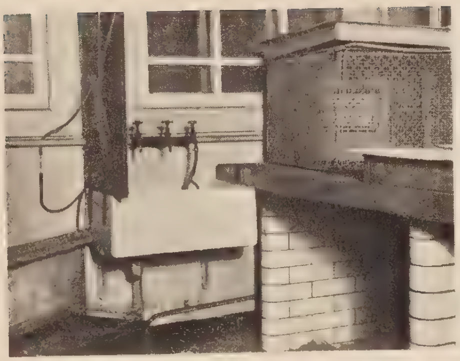

FIG. 2. Large glass tank, suction pump, trays containing water—one placed over electric fire.

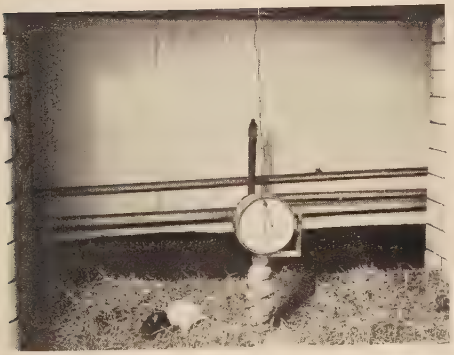

FIG. 3. Edney hair hygrometer. Sampling basket.

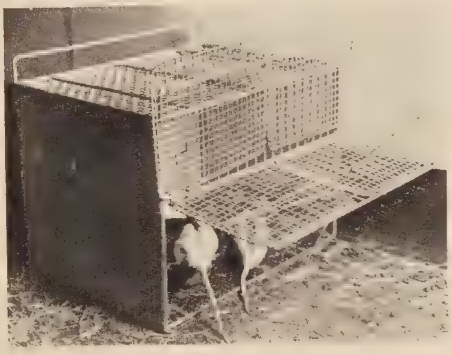

FIG. 4. Cage trap.

OBSERVATIONS ON MATING SCARS IN *GLOSSINA PALPALIS* (R.-D.).

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In a recent paper (Squire, 1950) attention was drawn to the presence of bilateral mating scars on the sternum of the sixth abdominal segment of mated females of *Glossina palpalis* (R.-D.). They vary from one or more blackened pin-points on the soft raised copulatory cushions of young females to gross black spots, in older much-mated females, which are easily visible to the naked eye. It is, therefore, all the more remarkable that these mating scars should have escaped the notice of so many observers in the past. Since this appears to be the case, however, judging by the absence of any reference to them in literature it may be worth considering the matter in some detail. To do this it is necessary to examine the male and female armatures and the mating habits of the fly.

The Male and Female Armature.

Following Newstead's terminology (1924), the male armature consists of the hypopygium hinged to the posterior margin of the seventh abdominal segment and folding forward ventrally over the seventh and sixth segments. On the inside of the hypopygium are mounted the superior claspers (fig. 1, s.c.), the inferior claspers (i.c.) and the penis with its vermiform appendices (v.a.). The female armature is simpler and comprises a sternal triangular plate (fig. 2, s.p.) attached at its base between the anus and the vulva and free at its apex ; and two anal plates (a.p.) astride the line of the anus (a.) and the vulva (v.) which are chitinised and raised with sharply defined inner margins. Below and anterior to the vulva there is a fold of skin (f.) which normally occludes this aperture. The function and coincidence of the male and female armature elements was determined by observation, from the outside of a large wire-mesh cage containing mating couples, by means of a hand lens ; and by

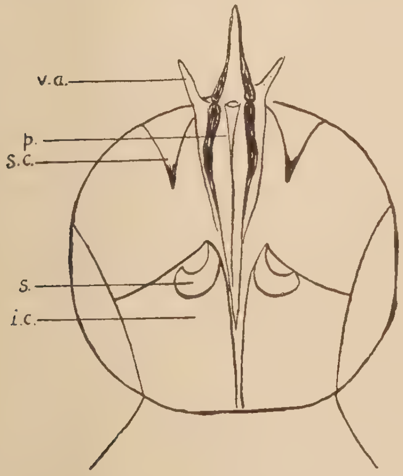

Fig. 1.—*Glossina palpalis* R.-D. Male armature extended backwards and seen from below.

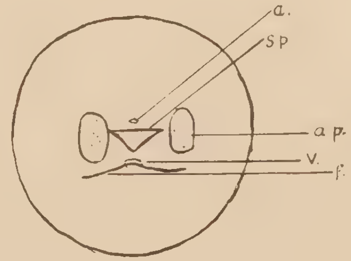

Fig. 2.—*Glossina palpalis* R.-D. Female armature, posterior view.

immersing copulating flies contained in wire-gauze cylinders in boiling water and examining the united armatures under a binocular microscope. In this manner it was ascertained that it is only the superior claspers which are applied to the mating cushions and that they are therefore solely responsible for the production of the scars. So far as can be ascertained from the literature available to the writer, the function of the curiously shaped inferior claspers has not been described before so that the following account will be of interest ; during copulation the outer edges of the scrolls (s) coincide with the inner margins of the anal plates and draw them apart horizontally. This has the effect of elevating the sternal plate which normally somewhat overhangs the vulva ; at the same time the fold occluding the vulva is drawn taut thus exposing that aperture. It may be of interest to remark here that in *G. longipalpis* where the anal plates are but feebly developed the inferior claspers also are reduced. The penis which is chitinised and normally folded upwards and backwards on itself is erected during copulation and is accompanied into the sac-like vulva by the vermiform appendages which are also erectile and whose functions are unknown. From the presence of chitinised spines all over their surface however it seems probable that at least one of their functions may be to keep the penis firmly anchored in the vulva.

Copulation.

This takes place at any time of the day but more especially after feeding. To obtain mating couples in the laboratory it is generally sufficient to give the flies a blood-meal. They stay *in copula* for a long time, sometimes for several hours and do not readily part even if they are disturbed. The male is very ardent. "*L'ardeur copulatrice*" according to Roubaud quoted by Hegh (1946) "*est tres grande*" but the female is often disinclined and can thwart the most determined male by simply keeping her wings closed and buzzing them when he attempts to mount. When the partners are attuned however conjugation takes place in a fraction of a second. The female opens her wings, the male mounts and grips the tip of her abdomen with the powerful hypopygium. The superior claspers normally folded backwards like a pair of fangs are erected and dug into the copulatory cushions on the sixth segment. There is generally a good deal of probing before the flies settle down. If they are disburbed the female readily takes flight carrying the male with her even though both flies be engorged. It was not possible to see for certain whether the male assists in these flights with his wings but it was thought not. Obviously in nature mating flies will be constantly molested by their numerous natural enemies so that they are not likely to accomplish so lengthy a performance without being disturbed many times. Flies mated in glass tubes usually remain quite still and there is but little probing or readjustment observed. In large cages however with other flies present and an observer armed with a hand lens in attendance, the female readily takes flight from point to point and this is usually followed by much activity of the hypopygium and claspers. It is not surprising then that scars often fail to develop when mating takes place in glass tubes. But there is another phenomenon of importance in the production of scars which seems to have attracted but little notice. In many cases a kind of orgasm takes place. The male becomes very excited, his middle and hind legs stroking the ventral side of the female's abdomen with great rapidity. Meanwhile the hypopygium keeps pumping, *i.e.*, opening and closing at the rate of about twice a second, and each time the falciform claspers are dug well into the mating cushions. This phase lasts for perhaps a minute and may be repeated after a quiescent interval. Here it may be of interest to draw attention to the absence of mating scars in the two other species of tsetse-flies found in Sierra Leone, *viz.*, *G. longipalpis* Wied. and *G. fusca* (Wlk.). In the former the claspers are reduced and in the latter the teeth are a good deal blunter than in *G. palpalis*. It is therefore possible that mating scars are confined to the *palpalis* group. As the same female may be mated many times in her life it is not difficult to account for the gross scarring of many individuals.

The Physiology of Scar Formation.

It was suggested to the writer that the blackening of the pits made by the points of the claspers may be due to the removal of the protective waxy layer on the surface of the skin by the action of the claspers and the consequent oxidation of the polyphenols contained in the epidermis in the manner demonstrated by Wigglesworth (1946) in regard to other insects. Owing to the lack of suitable reagents it was not possible to confirm this by experiment.

Significance of the Mating Scars.

Unscarred females are nearly always extremely teneral. From this it is concluded that mating takes place within a day or two of emergence. Roubaud (in Hegh, 1946) states that it occurs within a few hours. The percentage of unscarred females was therefore adopted as an index of the rate of emergence at any particular time. It was desirable however to determine the error which would almost certainly attach to such a method.

At the suggestion of Prof. P. A. Buxton in a letter to the writer this was done by spermatheca dissections. It was found that some females though showing no blackened pin-points yet bore pits in the mating cushions too obvious to be ignored. It was therefore decided to include these in the category of scarred females and in the following lines the term must be so understood. The dissections were made over a period of four months, July to October 1950, and the results are given in Table I. The flies were age-grouped as described by Squire (1950).

TABLE I.

	No. dissected	Spermatozoa	Ova	Larvae
AG1 Unscarred	37	5 (13·5%)	1 (2·7%)	— —
AG1 Scarred	116	109 (93·9%)	31 (26·7%)	1 (0·9%)
AG2 Scarred	87	86 (98·9%)	26 (29·9%)	5 (5·7%)
AG3 Scarred	40	38 (95%)	11 (27·5%)	5 (12·5%)

Conclusions.

From these figures it will be seen that 13·5 per cent. of the unscarred females had spermatozoa in the spermatheca. However, the low percentage of flies containing ova and the absence of larvae confirms the extreme youth of the flies and shows that those flies constituting the 13·5 per cent. had not long been mated. Thus it may be concluded that the absence of mating scars provides a fairly reliable index of the rate of emergence. In the scarred groups AG1, AG2, and AG3 only 6·1, 1·1, and 5 per cent., respectively, failed to show spermatozoa. These high rates of impregnation are accounted for not only by repeated mating but also by the efficient conservation of spermatozoa in the spermatheca. Females isolated from males for eighteen days were still found to have an abundance of active spermatozoa in their spermathecae. Owing, however, to the high mortality rate of flies in captivity, it was not possible to obtain extensive data. Attention should be drawn to the fact that although the larval rate is highest in AG3 both the ova and spermatozoa rates are lower than in AG2. This may possibly indicate a decline in the productivity of the oldest age-group.

Summary.

Attention is again drawn to the presence of mating scars in *G. palpalis*. They vary in degree and are caused by the superior claspers. The function of the inferior claspers is described and a function for the vermiform appendices is suggested. Flies mated under certain laboratory conditions may fail to develop scars, for reasons given. There are no mating scars in the two other tsetse-flies found in Sierra Leone, *viz.*, *G. longipalpis* and *G. fusca* owing to the character of the claspers in those species. For this reason it is likely that the scars are confined to flies of the *palpalis* group. A suggestion is made concerning the physiology of the production of the scars and attention is called to their bionomic importance. The results of spermatheca dissections of unscarred and scarred females are tabulated. From these it is concluded that the absence of mating scars provides a fairly reliable index of the rate of emergence at any particular time.

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THE ECOLOGY OF THE GARDEN CHAFER, *PHYLLOPERTHA HORTICOLA* (L.) WITH PRELIMINARY OBSERVATIONS ON CONTROL MEASURES.*

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(Plates XXII & XXIII.)

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The garden chafer has attracted the attention of entomologists for some considerable time. Curtis (1883) reported outbreaks in the Swansea district which occurred early in the 19th century, and Miss Ormerod (1893) in her reports described extensive injury to the foliage of fruit trees by adults and to grassland by the larvae. At the beginning of this century Warburton (1909, 1911) described its occurrence in Wales where it was abundant in hill pastures.

Taylor & Thompson (1928) described a chafer attack in Yorkshire, giving an account of the damage caused by adults and larvae and recommending the hand collection of adults as a means of control. They also found that dressings of crude naphthalene were effective in controlling the larvae.

Walton (1935) investigated an attack in Somerset and found that the adults preferred a meadow to an adjacent field which was closely grazed. Dressings of crude naphthalene appeared to be effective in controlling the larvae. Dressings of sulphur appeared to be ineffective in controlling the adults.

Bennett (1940) tested the effect of various chemicals on the larvae but concluded that none were effective at economic concentrations or at concentrations which did not damage the turf.

Thomas & Heal (1944) described the damage caused to grassland in North Wales in 1942–3 by the larvae of *P. horticola* (L.) and the Welsh chafer *Hoplia philanthus* Fuess. They selectively sampled a number of fields with populations ranging from 0–950,000 third-instar larvae per acre and showed that the damaged parts of the fields carried higher populations than the undamaged parts, though some undamaged areas carried populations up to 600,000 larvae per acre. They compared the life history and morphology of the larvae and pupae of *P. horticola* with that of *H. philanthus* and showed that all the stages of *H. philanthus* occurred later than those of *P. horticola*.

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Since the work described in the present paper was begun, Gray & others (1947) have given some details of the occurrence of *P. horticola* in the Lake District between 1935 and 1939, describing its life history and the results of preliminary investigations on control.

On the Continent, and in America, more attention has been paid to other chafers of economic importance. One of the most extensive papers on *P. horticola,* however, is that of Rittershaus (1927). She described in detail the morphology of all the stages of *P. horticola.* Her observations on the behaviour of the adults in the Berlin area in 1924–5 are particularly detailed. The behaviour of the beetles on emergence, their mating and dispersal, are carefully described. Adults were mated in the laboratory and the behaviour of the female during oviposition was observed. The data on the number of eggs laid are scanty but it was shown that adults could mate and lay fertile eggs without feeding. The developmental stages are described in detail.

With the exception of Rittershaus the early investigators were concerned primarily with the control of *P. horticola* by means of insecticides and repellents. The biology and ecology of the insect received little attention. In the more recent work of Thomas & Heal and Gray, attention has still been focussed on control and economic importance, but some advance has been made in understanding the relationship between the insect and its environment. Their work emphasises the need for further study of this relationship.

The work to be described in the present paper had for its scope a field study in the Bristol area of the biology of the garden chafer *P. horticola.* This study had a dual purpose. Firstly, to extend our present knowledge of the biology of the insect and in particular to discover environmental factors which affect its distribution and abundance ; secondly, to investigate the effect of the insect, and particularly the larvae, on the environment. In a study of this scope much of the work is of necessity exploratory, serving rather to break the ground and reveal those aspects of the problem which would repay further study, than providing a detailed investigation of particular points.

Brief Statement of Life-Cycle of *P. horticola.*

P. horticola has an annual life cycle. The adults (Pl. XXII, fig. 1) appear in May or early June and have a flight period lasting three or four weeks. The eggs (Pl. XXII, fig. 2) are laid in the soil, usually of grassland, and the larvae live in the surface soil feeding on the grass roots (Pl. XXII, fig. 3).

As a result of feeding, the grasses may be destroyed and damaged patches of turf may be seen by the autumn (Pl. XXIII). There are three larval instars, the third (Pl. XXII, fig. 4) being reached by early autumn. Feeding ceases in the winter and the larvae hibernate until the following spring. After a short prepupal stage the larva ecdyses and the pupa is formed within the cast-off larval skin (Pl. XXII, fig. 5). The pupal stage lasts about a month.

Geographical Distribution and Habitat in England and Wales.

P. horticola is widely distributed in England and Wales though it is more abundant in the western half of the country. It is to be found extensively in the Lake District, North Wales, the Cotswolds, and the Dorset Downs (Pl. XXIII). Reports of its occurrence in other parts of the country are frequent, and attacks have occurred in Yorkshire, Cheshire, Lancashire, Shropshire, Berkshire, Essex, Surrey, Somerset, Devon, mid and south Wales.

The areas in which it is endemic have a number of features in common. There is a relatively high rainfall, the land is hilly and the infested areas are usually on the slopes. The infested grassland is poor quality permanent pasture and the soil is invariably light. Where outbreaks occur in other parts of the country, some or all of these features are found to characterise the infested area.

The work embodied in this paper was carried out in the south-west region in the counties of Gloucestershire, Wiltshire, Somerset and Dorset, and so a more detailed account of the distribution of *P. horticola* in this region is possible.

In Gloucestershire, the main district infested is the south-western face of the Cotswolds, including the Minchinhampton Common and the land around Wotton-under-Edge. In 1947 a single outbreak was reported from the Berkeley Vale near Thornbury, and was noteworthy in that the infested pasture was in the valley, and the soil, though fairly light, was heavier than in the other areas studied.

The garden chafer is not common in Wiltshire, but between 1944 and 1948 sporadic outbreaks occurred on lawns in the Chippenham area.

In Somerset, the most heavily infested locality is the Tickenham Ridge, running between Bristol and Clevedon. Further south, near Yeovil, some fields on a single farm were known to be infested.

The most extensive infested region is to be found on the Downland in Dorset. Three main districts are affected : a western district covering the parishes of Long Bredy, Lytton Cheney and Askerswell ; a central district covering the parishes of West Steepleton, Portisham and Frampton, and a northern district covering the parish of Alton Pancras. Within these districts it was estimated that, in 1947, at least 700 acres were infested.

The annual rainfall of the south-west region which includes all the infested areas is between 30 and 40 ins. In the Cotswolds and on the Tickenham Ridge the soil is light, overlying limestone brash, and in places only 3 or 4 ins. deep. In Dorset the soil is light, overlying the chalk, and of greater depth. Drainage in all the areas is good.

The vegetation will be discussed in detail later, but the great majority of the infested fields are permanent grassland which is of poor quality and used chiefly for grazing. In Dorset, some temporary grassland is infested and this is usually to be found on the lower slopes of the Downs, below rougher pastures which are heavily infested and from which the beetles have migrated. The fields invariably contain a large number of species of flowering plants of which a high proportion are weeds.

Within the districts in which it is endemic, the distribution of *P. horticola* is markedly local. It is quite usual to find one pasture heavily infested and the adjacent field free of beetles and larvae. Even within a field the distribution is irregular. In Ogbourn's field, on the Tickenham Ridge, two patches, each about 3 acres in extent, were heavily infested, but in the rest of the field only occasional larvae were found.

The distribution of Cadbury Camp (Tickenham Ridge, Somerset), and Alton Pancras (Dorset), was similarly irregular.

The Areas Covered by the Present Work.

Most of the areas infested by *P. horticola* in south-west England have been visited, but certain aspects of the work have been studied in detail in three localities. These localities are as follows :—

The Tickenham Ridge, Somerset.

Detailed studies have been made at two places on the Tickenham Ridge :

(*a*) Ogbourn's field ; a permanent grass field of about 12 acres on Naish Farm, Clapton-in-Gordano. The field is grazed during most of the year, but is shut up for hay during the summer. Part of the field had been infested with *P. horticola* for some years before the present investigations were begun.

(*b*) Cadbury Camp ; an extensive stretch of common land about two miles west of Naish Farm, used only for rough grazing. *P. horticola* is to be found over most of the common but investigations were restricted to an area of about 1½ acres which was heavily infested.

Lytton Cheney, Dorset.

The area studied at Lytton Cheney was part of the Downland on the farm of Colonel Wordsworth, Baglake, Lytton Cheney. The Downland which in this locality rises from about 300 feet to just above 400 feet above sea level, is thrown up into folds to form a series of narrow valleys or coombs, running from north to south. Investigations were carried out in one of these coombs which was about 20 acres in extent and grazed throughout the year. The coomb had been infested for some years before the investigations were begun.

Alton Pancras, Dorset.

Investigations were carried out in two fields on the farm of Mr. Waterman, Barcombe Farm, Alton Pancras. Both fields were permanent grassland about 400 feet above sea level, on the light soil overlying the chalk. One field was grazed in the spring and autumn and shut up for hay during the summer. The other, where the grass was poorer, was grazed at intervals throughout the year. Both fields were infested when the investigations were begun.

More detailed accounts of these areas will be given where necessary in the text.

Biology of the Adults.

Emergence.

Rittershaus (1927) described the emergence of adults as it occurred near Berlin in 1924–25. She records that usually adults appeared in early June, but that emergence depended on the weather and in mild seasons adults were known to appear and lay eggs in May. Emergence began about 9 a.m. The beetles crept out of the ground, clung for a while to the grass stalks and then flew away. This mass emergence only took place on four days and on each day the main emergence was between 9 a.m. and 10 a.m. By mid-day the adults were widely scattered.

In Britain, Taylor & Thompson (1928) record that in Yorkshire the larvae pupated in May, beetles were seen on 8th June and became numerous during the following two or three weeks. Thomas & Heal (1944) give the earliest record of *P. horticola* in North Wales as 21st May when three beetles were seen, but by 31st May they were swarming. Gray (1947) described a mass emergence of beetles occurring in June in the Lake District.

Near Bristol and Dorchester, where observations have been made for four and two years respectively, emergence has tended to be earlier than in other parts of the country. In 1945, the first beetles were found on the Tickenham Ridge on 9th May and were abundant by 15th May when mating was observed. In the same year Mr. Bainbrigge Fletcher reported finding *P. horticola* for the first time on 9th May on Rodborough Common in Gloucestershire. Emergence was later in 1946 when beetles appeared on 20th May at Tickenham and were abundant by the beginning of June. After the exceptionally severe winter of 1946–7 the first beetles were seen on 31st May at Bracken Hill and Tickenham, Bristol, and at Lytton Cheney near Dorchester. In 1948 emergence began at Bristol and near Dorchester on 17th May.

Up to 1948, one of the striking features about emergence in south-west England was that it occurred on or about the same date at places separated by as much as 70 miles. In 1948, however, emergence was more erratic. It began at Bracken Hill, Tickenham and Lytton Cheney on 17th May, but at a farm at Dunkirk, Gloucestershire, 20 miles from Bristol, beetles were not seen until 28th May. This difference may have been due to the changeable weather at the time. A period of cold weather began at Bristol on 22nd May and lasted for about five days. During this time no beetles were seen at Bracken Hill or at Tickenham, but they reappeared with the fine warm weather on 27th May. Emergence at Dunkirk coincided with this second period of activity at Bristol. The coincidence of emergence at Bracken Hill, Tickenham, Lytton Cheney and Rodborough Common (on the one occasion when emergence was reported), may be owing to the similarity of the sites. In each case the infested areas are about 300 feet above sea level and have a southerly aspect. Where there are marked differences between the sites as, for example, in the Lake District, the dates of emergence vary appreciably. (Gray, 1947.)

A characteristic feature of emergence is the abruptness with which it begins. For a few days occasional beetles may be seen, then quite suddenly there is a mass emergence of beetles which invariably begins in the morning, usually about 10 a.m. For some days before the main emergence, fully formed adults may be found lying quiescent in the soil, often with the last larval skin still surrounding them. Rittershaus and Harper Gray have also remarked on the abruptness of emergence.

In the years 1945–8 males invariably appeared before females. In 1945, although beetles were abundant from 15th May and occasional pairs were seen mating, it was not until 23rd May that pairs were plentiful ; a similar sequence of events was observed in 1946. In 1947, beetles were collected daily from 31st May when emergence began. On each of the first four days of the flight period 100 beetles were collected at random. The percentage of females each day was 5, 7, 3 and 8 per cent. By 6th June, females were plentiful and 25 mating pairs were picked up in a few minutes. On 9th June, 30 per cent. of the beetles caught were females. In 1948, 100 beetles were collected from the garden at Bracken Hill each day they were flying. As the garden was small, the beetles were liberated after they were sexed. During the first four days the percentage of females caught was 4, 5, 2 and 4 ; the fifth day was dull and only three males were caught but on the sixth day the percentage of females rose to 13. There followed five days of dull, wet weather when no beetles were seen. Thereafter, few beetles were seen and a reliable estimate of the percentage of females became impossible. Apparently the unfavourable weather coincided with the time when the females were beginning to emerge in fair numbers. As the sexes emerge at different times, the weather may be an important factor in determining the extent of mating. It is interesting that in the autumn of 1948 no signs of damage by larvae were seen on the lawns at Bracken Hill, whereas in 1947 there had been appreciable damage.

The Flight Period.

Activity on the first day of mass emergence usually lasts about an hour. The beetles crawl about in the grass if the weather is dull and fly only if the sun is shining. Under similar weather conditions, the beetles are active for a longer period each day of the flight period. Although the temperature and sunlight may be as great during the afternoon as in the morning, activity declines and it is only when the flight period is well advanced that the beetles are active to any extent after 2 p.m.

The weather generally was dull and warm at Tickenham during the flight period in 1945. For the first few days at Ogbourn's field, the beetles were to be found among the herbage in the infested part of the field. Flights were of short duration restricted to the short periods of sunshine. It was a remarkable sight to see the beetles suddenly take wing and circle low over the field when the sun broke through the clouds, only to settle again just as suddenly when the sun was obscured. It is characteristic of the beetles that they do not spread far from their place of origin and this natural inclination was accentuated by the dull weather. Only occasional beetles were seen in the adjoining field of oats and in the part of Ogbourn's field beyond the infested area. On this point my observations differ from those of Rittershaus. After some days, however, large numbers of beetles were seen on the brambles in the hedge and on the leaves of the lower branches of the trees in a small wood across the road.

In 1946, although there was more rain during the flight period than in 1945, there were longer periods of sunshine. More extensive flights were made and beetles were found up to 50 yds. into the adjoining field which was then in a clover ley. Some eggs were subsequently found in some soil samples taken from the ley.

Mating, which has been described in detail by Rittershaus (*loc. cit.*), begins soon after the females emerge. The male climbs on the back of the female and is carried round by her for some time. Copulation no doubt occurs during the period of activity in the forenoon, but pairs may be found in the hedge late in the day. As males will attempt to copulate with each other and may be found in groups of even three or four on top of one another, it is only by sexing the pairs that one can be sure that mating is taking place.

After mating, the females enter the soil for oviposition. Towards the end of the flight period, which usually lasts about three weeks, most of the beetles found were females, though this may be because they remain near the oviposition sites while the males are more widely dispersed. However, in laboratory experiments with pairs of adults, it was found that the male usually dies before the female and often needs to be replaced a second or third time before the female dies. These observations differ from those of Gray who states that males live longer than females.

At Lytton Cheney where the flight period was observed during 1947 and 1948, the general sequence of events was similar to that at Tickenham. On the whole, there were longer periods of sunshine and the flights of the beetles were longer in relation to their daily period of activity. The grass in the infested coombs was shorter than in Ogbourn's field and the beetles tended to re-enter the soil when it was dull instead of remaining active in the grass. After some days large numbers of beetles were found feeding or resting in the hedge running along the bottom of the coombs. It would seem that in the south-west a nearby hedge, especially if it contains blackberry or dog rose, attracts the beetles in the way that bracken does in Wales and the Lake District. In the south-west the salad burnet, *Poterium sanguisorba*, is common in the infested fields and is a favoured food plant of the adults which may be found in numbers feeding on the leaves.

Oviposition.

In order to obtain information on the number of eggs laid throughout the oviposition period, 22 females were mated and the eggs laid by each one were collected. The beetles were collected from Ogbourn's field at the beginning of the flight period in 1945 or were reared from larvae kept in the insectary through the winter. The pairs were kept in 2 lb. jam jars containing about 1 inch of moist soil which had been put through a 2 mm. sieve. Leaves of blackberry and dog rose were supplied for food and were removed when they had wilted or had been eaten. To begin with, the jars had been covered with butter muslin but as the soil in them dried out rather quickly, the muslin was replaced by glass plates arranged to allow an air passage but to reduce evaporation. Every other day the soil was sifted and the eggs removed. If the male had died it was replaced by another. The results of this experiment are summarised in Table I.

The 22 females laid a total of 292 eggs, an average of 13·3 eggs per female. The number of eggs laid by each female varied from 1 to 33. The behaviour of the females after copulation varied considerably. In general, up to six days elapsed before eggs were laid. Egg laying continued at intervals for about ten days and the female died soon after the last eggs were laid. During the oviposition period, the females spent a good deal of time in the soil, though they were often seen on the surface, resting or feeding on the leaves. In some cases, copulation was observed to occur two or three times. Usually the females died in the soil but the males were more frequently found dead on the surface. So far as could be judged from sieving the soil, the eggs were laid singly and scattered about in the soil.

TABLE I.

Oviposition expt. 1945.
Numbers of eggs laid by individual females.

Pair No. ...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
22nd May...	–	–	–	–																		
23rd ,, ...	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
24th ,, ...	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
26th ,, ...	8	–	–	–	–	3	15	22	19	–	–	–	–	–	–	–	–	–	–	–	–	–
28th ,, ...	8	4	4	2	8	30	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
30th ,, ...	2	1	8	3	1	–			–	–	1	–	1	1	–	–	–	–	–	–	8	–
31st ,, ...	–	–	–	–	–					–	–	–	–	–	–	–	–	–	–	–	–	–
2nd June...	–	3	–	2	–					–	–	–	9	1	–	1	–	10	–	3	7	–
6th ,, ...	1	–	–	–	7					–	–	1	–	1	–	5	1	–	2	4	2	19
8th ,, ...	–	2	–	2	–					–		10	–	4	6	–	1		5		–	4
10th ,, ...										–		–		–	6		3		8			
13th ,, ...										8		–					2					
18th ,, ...										–		–										
Total laid ...	22	10	12	9	16	33	15	22	19	8	1	11	10	7	12	6	7	10	15	7	17	23

Total = 292 Mean = 13·3.

Table I shows that the behaviour of the females from pairs 6, 7, 8 and 9 was markedly different from the general pattern. These females laid 33, 15, 22 and 19 eggs respectively in 2–4 days. The previous history of these particular beetles may account for their peculiar behaviour, for they were reared from larvae which had been kept in tubes in the insectary during the winter and had emerged some considerable time before they were mated. There can be little doubt that during the long period between emergence and mating all the eggs had matured and were ready to be laid as soon as mating occurred. Normally mating occurs soon after emergence and the eggs are laid a few at a time, as they mature, until oviposition ceases.

These data amplify those of Rittershaus and Gray. Rittershaus recorded that four females laid 11, 36, 27 and 16 eggs respectively. Gray dissected 20 females and found an average of 12 eggs in the ovaries with a range of 6 to 17. It is not clear, however, whether or not these females had already laid some eggs before the dissections were made.

Relation between Weight of Adults and Fecundity.

The larvae from which females 6, 7, 8 and 9 were reared had been weighed during the winter of 1944–5. Feeding had then stopped and the gut was empty. The weight of the larvae and the number of eggs laid by the corresponding adult was as follows :— Weight of larvae, 0·158, 0·184, 0·222 and 0·243 gm. ; number of eggs laid by the respective adults, 15, 19, 22 and 33.

There is a striking correlation between the larval weight and the number of eggs in these four instances but it was decided to repeat the experiment in 1946 using larger numbers of adults.

Pupae were collected from Ogbourn's field on 8th May 1946. They were placed together in moist soil for 48 hours and then weighed and kept in separate tubes until the adults emerged. Each female was mated with a male from the same series of pupae and the pairs set up in oviposition jars as in 1945. The males were selected at random and if the male died before the female another was put in the jar. The data from this experiment are summarised in Table II.

Five hundred and fifty two eggs were laid by the 38 females ; an average of 14·5 per female which corresponds well with 13·3 in 1945 considering that in 1945 the females were a random selection while in 1946 they were chosen to give a wide range of pupal weight. The oviposition pattern was essentially similar to that in 1945.

There is a significant correlation between the body weight of the pupa and the number of eggs laid by the adult, "r"=·380, 5% value=·325 for 36 d.f. Clearly the fecundity of the female is directly related to its weight. As the larvae do not feed after hibernation, one can assume that fecundity is directly related to the food reserves of the larvae at the beginning of hibernation.

Some estimate of the extent to which fecundity is limited by the weight of the larvae may be obtained by comparing the weight of the heaviest and lightest adults in the oviposition experiment. The 19 heaviest adults, from pupae of average weight ·235 gm., laid 316 eggs, an average of 16·6 per female. The 19 lightest adults from pupae of average weight ·183 gm., laid 236 eggs, an average of 12·4 per female. Thus a difference of approximately ·05 gm. average weight affects the fecundity by as much as 25 per cent.

Natural Limitation of the Weight of the Larvae.

The dependence of the fecundity of the females upon the weight of the larvae makes it important to know whether there are any environmental factors that limit the weight of the larvae. As will be shown later, the damage which the larvae cause to the turf by feeding on the grass roots can be very severe. By early autumn, the turf in heavily infested areas may be completely destroyed and it is evident that there

TABLE II.

Oviposition expt. 1946.
Number of eggs laid by individual females derived from weighed pupae.

Pair No. ...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Pupal weight of ♀ in mg.	197	199	158	230	208	194	207	212	217	164	190	179	187	192	245	199	229	238	210
Mated ...	20.v.1946												29.v.1946						
31st May ...	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1st June ...	–	–	–	–	–	–	5	1	1	3	–	11	7	–	–	1	–	–	–
3rd ,, ...	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4th ,, ...	–	–	–	–	–	–	–	1	–	–	9	–	–	–	–	–	3	–	–
6th ,, ...	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7th ,, ...	5	–	–	–	–	–	16	1	–	–	–	–	9	16	–	16	18	–	–
8th ,, ...	–	–			–	–	–	–		–			–	–	–	–			–
9th ,, ...	–	–			9	–	–	1		1			1	–	–	–			9
10th ,, ...	–	–			–		–	–		–			–	–	–	–			–
13th ,, ...	4	–			–		–	–		10			3	–	3	2			4
15th ,, ...	–	–								–			3	2	9	14			8
17th ,, ...		–								–				1		–			–
19th ,, ...																–			
20th ,, ...																–			
21st ,, ...																–			
23rd ,, ...																2			
Totals ...	13	–	2	–	9	–	21	4	1	14	9	11	23	19	12	35	21	–	21

Pair No. ...	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
Pupal weight of ♀ in mg.	218	236	205	245	263	240	259	257	181	180	253	169	169	148	170	279	199	219	187
Mated ...											2.vi.1946								
31st May ...	–	–	–	–															
1st June ...	–	–	–	–															
3rd ,, ...	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4th ,, ...	2	4	17	2	17	–	7	10	2	–	–	–	–	–	–	–	–	–	–
6th ,, ...	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
7th ,, ...	16	25	2	18	15	1	19	1	3	–	–	8	–	–	7	27	3	–	–
8th ,, ...	–	–	–	–	–	–	–	–			–	–	–	–	–	–	–	–	6
9th ,, ...	–	–	–	–	–	–	–	–			–	–	–	–	4	–	–	–	–
10th ,, ...	–	–	–	–	–	–	–	1			–	–	–	–	–		–	–	–
13th ,, ...	–	–	4	6	1	–	–	–			10		–	9	7		–	–	
15th ,, ...		–	9		4	–	–				–		–	1	–		–	–	
17th ,, ...		–	–			–					8		–	–	–		18	5	
19th ,, ...		6	–			11							–	–			–		
20th ,, ...		–	–											–			–		
21st ,, ...		3	–														–		
23rd ,, ...		8																	
Totals ...	18	46	32	26	37	12	26	12	5		18	8		10	18	27	21	5	16

Total = 552 Mean = 14.5 "r" = ·380 5 per cent. value = ·325 for 36 d.f.

TABLE III.

Effect of feeding upon fecundity.
Number of eggs produced by individual weighed females.

Group A.—Adults fed.

Pair No. ...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Daily Total		Total Infertile
Weight of ♀ (mg.) ...	64	73	75	77	77	82	86	93	97	100	101	109	110	111	112	128	134			
8th June...	1	–	–	–	–	–	–	–	–	–	–	10	1	–	–	–	–	12		–
9th ,, ...	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–
10th ,, ...	–	10	2	6 (5)	6	1	–	17	–	4	–	11	1	–	–	–	–	58		5
11th ,, ...		–		1 (1)	–	13	2	–	–	4	–	–	2	–	–	–	5	27		1
12th ,, ...				–	–	1	–	–	–	9	1	–	11	–	–	–	6	28		–
13th ,, ...					2		–		–	–	–	–	–	–	5	–	1	8		–
14th ,, ...					–		–		–	–	–	–	–	–	1	–	–	1		–
15th ,, ...					2 (1)		–		–	–	–	–	–	–	7	–	6	15		1
16th ,, ...					–		–		3		–	–	–	–	1	2 (2)	–	6		2
17th ,, ...							–		12 (1)		–	–	–	–	–		–	12		1
18th ,, ...							–		4 (1)		3	–	–	4 (2)	–		–	11		3
19th ,, ...											–	1	–	2	–			3		–
20th ,, ...											2	–	1	5 (5)	–			8		5
21st ,, ...											–	–	1		–			1		–
22nd ,, ...											–	–	–		–			–		–
23rd ,, ...											–	–	6		–			6		–
24th ,, ...											6 (4)	–	–		–			6		4
25th ,, ...											1 (1)	–	–		–			1		1
26th ,, ...											6 (5)		–		1 (1)			7		6
27th ,, ...											1 (1)		–		15			16		1
28th ,, ...											–		–		2 (1)			2		1
Eggs Laid...	1	10	2	7	10	15	2	17	19	17	20	22	23	11	32	2	18	228	mean 13·4	31
Residual ...	7	–	12	5	–	–	12	–	3	–	–	–	–	12	1	20	–	72		
Total Eggs	8	10	14	12	10	15	14	17	22	17	20	22	23	23	33	22	18	300	mean 17·6	

Figures in brackets denote number of eggs laid that were infertile.

TABLE 111—*cont.*

Group B.—Adults not fed.

Pair No. ...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Daily Total		Total Infertile
Weight of ♀ (mg.) ...	65	70	73	75	76	79	80	83	93	97	102	103	110	111	116	118	120	128	129	156			
8th June...	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–
9th ,, ...	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–
10th ,, ...	–	–	2	3	5	9	10 (1)	–	11	–	–	–	–	–	–	–	–	–	–	6	46		1
11th ,, ...	1	–	5	1	1	–	2	2	1	–	–	1	–	–	–	–	–	–	–	–	14		–
12th ,, ...	5	–	–	4	–			10	–	15		6	–	–	–	10	–	2	1	13	66		–
13th ,, ...	–	–	–	–	–			–	–	–		–	–		4 (1)	12 (2)		–	4	–	20		3
14th ,, ...	–		–	–	–			–	–	2 (1)		–	–		–	–		4	–	11	17		1
15th ,, ...	–		–	–	–			–	–	–			–		5	2 (1)		21 (1)	15 (2)	2	45		4
16th ,, ...			–	–	–				–	–			–		10 (1)	1 (1)		–	–	–	11		2
17th ,, ...				–	–				–	–			–		7 (1)			–	–	–	7		1
18th ,, ...				–	–				–				1 (1)		–			–	–	–	1		1
19th ,, ...				–					–											–	–		–
Eggs Laid...	6	–	7	8	6	9	12	12	12	17	–	7	1	–	26	25	–	27	20	32	227	mean 11·4	13
Residual ...	3	12	–	–	–	3	–	–	–	–	18	12	13	17	1	1	23	–	2	–	105		
Total Eggs	9	12	7	8	6	12	12	12	12	17	18	19	14	17	27	26	23	27	22	32	332	mean 16·6	

Figures in brackets denote number of eggs laid that were infertile.

must be greater competition for food among larvae in these areas than in those where the turf is not damaged. Investigation has shown this to be so. The average weight of 56 larvae collected from severely damaged parts of Ogbourn's field in the winter of 1944 was ·178 gm. compared with ·251 gm. for 11 larvae from undamaged parts. Further data were obtained in the spring of 1946. The average weight of 59 pupae from undamaged parts of Ogbourn's field was ·207 gm. compared with ·173 gm. for 42 pupae from damaged parts of the field, a significant difference. (t=5·59. 1 per cent. value=2·627 for 99 d.f.)

Clearly the weight of the larvae may be limited if the food supply is exhausted before the end of the feeding period and the extent to which this natural limitation may effect the fecundity of the females may be seen by comparing the result with the 1946 oviposition experiment. There a difference of ·05 gm. corresponded to a difference in fecundity of 25 per cent. The difference of ·034 gm. observed in the field corresponds to a difference of 17 per cent.

Effect of Adult Feeding upon Fecundity.

In 1946, Rittershaus' observation that females could lay fertile eggs without feeding was confirmed. Three females reared from pupae kept in the insectary laid 1, 22 and 19 eggs respectively, though neither they nor the males with which they were mated fed during their adult life. The eggs developed normally.

In order to find out whether feeding by the adults affected their fecundity, two experiments were carried out in 1947 and 1948. In the first experiment there were two groups of adults, A and B ; Group A was fed on a mixture of grasses, bracken and burnet, group B received no food but the pairs were given strands of wood wool to take the place of foliage for climbing or resting. In the second experiment there were five groups which were offered bracken, blackberry, burnet, grass and no food respectively.

The beetles for these experiments came from pupae collected from the field just prior to emergence. The adults were practically fully formed and were kept in a tin of moist soil until they attained their full colour and just became active. They were then sexed, weighed and the females divided into groups so that in each experiment the groups had as nearly as possible the same range and mean body weight.

In the first experiment the mating jars were examined each day for eggs but owing to lack of time the mating jars in the second experiment were examined on only three occasions. The eggs obtained were incubated to determine those which were infertile and, at the end of the experiments, each female was dissected to determine the number of eggs remaining in the ovaries and oviducts.

The data from these two experiments are recorded in Tables III and IV.

In the first experiment, the 17 females in group A laid 228 eggs, an average of 13·4 per female. The 20 females in group B laid 227 eggs, an average of 11·4 per female (Table III). An analysis of covariance shows that there is a highly significant correlation between body weight and fecundity, but that the difference of 2·0 between the means of group A and group B is not significant. (Correlation coefficient r=·529. 1 per cent. value for 34 d.f.=·425. Variance ratio F=1·12. 5 per cent. value=4·13.) There is therefore no evidence that feeding in the adult stage significantly affects fecundity.

For the second experiment (Table IV) an analysis of covariance shows that there is no significant difference between the number of eggs laid by the groups receiving bracken, blackberry, grass and no food. The difference between the number of eggs laid by the group receiving burnet and the remaining groups is highly significant. (t=3·866. 1 per cent. value=2·724 for 34 d.f.) Burnet evidently increases the fecundity of the adults, but bracken, blackberry and grass do not.

It is difficult to reconcile the results from these two experiments and the need for further investigation of the effect of feeding on oviposition is evident.

Field observations have shown that burnet is a favoured food plant for, when it occurs in infested fields, adults are readily found feeding on it. It was, moreover, present in small quantities in the food given to the adults in the first experiment.

Other points of interest emerge from these two experiments. In group A in the first experiment there is a positive correlation between body weight and length of life which is significant at the 5 per cent. level (r=·598. 5 per cent. value=·482). In group B the correlation is not significant (r=·122. 5 per cent. value=·44).

The average number of days over which eggs were laid was 4·75 in group A and 2·3 in group B. Analysis shows this difference to be significant. (Variance ratio F=5·05. 5 per cent. value=4·13.)

A difference of behaviour in response to the treatments may account for this, for it was observed that the beetles which were fed spent more time on the surface feeding or resting in the foliage than those which had only wood wool to move in.

It is noteworthy that at least three of the adults in group A, Nos. 11, 13 and 15, laid two distinct batches of eggs. A similar oviposition pattern was noted among some of the heavier adults in the 1946 experiment. These results disagree with the statement of Gray (1947) that only one series of eggs is laid. Unfortunately he did not give the data on which his statement was based.

Fertility.

Fertility refers to the viability of the eggs laid. In 1947 and 1948 the eggs laid by each female each day were kept on moist soil in small tins with tight fitting lids which were kept in a saturated atmosphere. When the eggs hatched the larvae burrowed into the soil and were subsequently recovered. The infertile eggs, which usually began to be attacked by mould after some days, were removed at intervals. In the first experiment (Table III), 455 eggs were laid, 411 were fertile and 44 or 9·7 per cent. were not. Fig. 1 shows that, while the daily total of eggs laid rapidly reached a maximum and then fell slowly during the rest of the oviposition period, the number of infertile eggs remained fairly constant. Fertility evidently decreases as the age of the female increases.

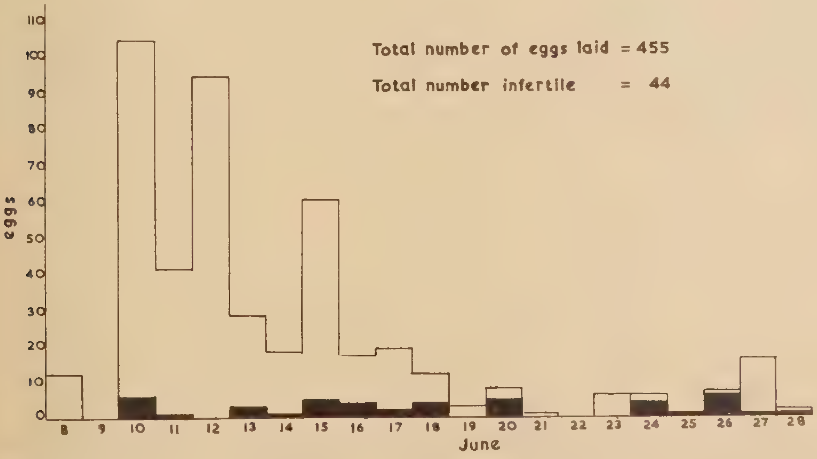


Fig. 1.—Daily total of eggs laid; the shaded portion indicates infertile eggs. Figures derived from Table III.

When the two groups are considered separately it will be seen that there is a difference in fertility. Group A laid 228 eggs of which 31 or 13·6 per cent. were infertile, group B laid 227 eggs of which 13 or 5·7 per cent. were infertile. The difference is significant. ($X^2=7·06$. 1 per cent. value$=6·64$.) Examination of the data shows that this is entirely owing to the longer oviposition period of the adults in Group A, for if we compare the fertility of the eggs laid up to June 17th we find Group A laid 167 eggs of which 10 or 6·0 per cent. were infertile and group B laid 226 eggs of which 12 or 5·3 per cent. were infertile.

The results of the second experiment (Table IV) confirm those of the first. Of the 752 eggs laid, 40 or 5·3 per cent. were infertile and the proportion of infertile eggs increased in successive periods of the experiment.

Residual Eggs.

On dissecting the females for residual eggs, it was found that for the most part the ovaries had degenerated and the eggs were lying in the oviducts which were sometimes greatly distended by them. Not one of the beetles was completely barren and the total of eggs is more closely correlated with weight than is the number of eggs laid.

In the first experiment the average number of eggs produced by Group A was 17·6 compared with 16·6 for Group B. The difference is not significant. (Variance ratio $F=2·68$. 5 per cent. value$=4·13$.)

An interesting feature of the second experiment is that there were no residual eggs in the beetles fed on burnet. An analysis of covariance shows that the average number of eggs produced by these beetles was significantly greater than the average for the other groups. ($t=3·87$. 1 per cent. value$=2·72$.) Evidently there were two differences between the beetles fed on burnet and the others ; firstly, the burnet group produced more eggs and, secondly, a higher proportion of those eggs was laid.

Incubation Period.

In 1945, the eggs collected each day from the oviposition experiments were placed on moist soil in petri dishes and incubated at 18°C. The results are presented in fig. 2 in the form of a histogram. The distribution curve is skewed and shows the average incubation period to be 22–25 days, though it may be as short as 20 days or as long as 30 days. This considerable range may have been partly due to variations in humidity in the petri dishes. The eggs absorb water, increase in size and change shape appreciably during development and it was noticed in subsequent years that, if the soil on which the eggs were incubated dried out to any extent, the eggs no longer increased in size and development was delayed. It was convenient to incubate the eggs on the surface of moist soil so that one could readily see when each egg hatched.

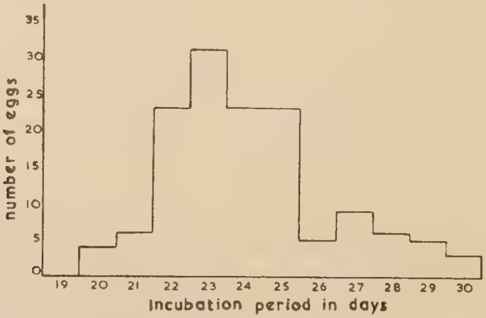


Fig. 2.—Incubation period of eggs of *P. horticola* at 18°C.

By using small tins with tight fitting lids kept in a saturated atmosphere in a desiccator, the risk of partial desiccation was reduced to a minimum.

The incubation period in the field is undoubtedly greater than 22 days and observations from field sampling in the present work confirm those of Gray that it is approximately 28 days. This difference is thought to be due to a lower temperature rather than to a humidity deficiency in the field, though it may well be that both factors play a part.

FIELD POPULATIONS OF EGGS AND LARVAE.

The work to be described in this section concerns the numbers of eggs and larvae found in the field, their spatial distribution and seasonal changes in abundance.

The Area Studied and Methods Used.

The spatial distribution and seasonal change in population of *P. horticola* have been studied at Ogbourn's field on the Tickenham Ridge. Ogbourn's field (fig. 3) is a permanent pasture grazed in the early spring and autumn by a small herd of dairy cattle and shut up for hay during the summer. It is bounded on the south and east by a narrow road lined with bushes and young trees. On the north, it is separated by a fence from a pasture which is grazed throughout the year and to the west by a cart road from an old arable field which in 1945 carried a crop of oats undersown with a ley mixture. This ley, which has remained down to the present time, has been grazed in the autumn and spring and cropped for hay in summer. Across the road to the south of Ogbourn's field is a small mixed deciduous wood.

A preliminary sampling in the autumn of 1944 showed that two parts of Ogbourn's field were infested by larvae of *P. horticola*.

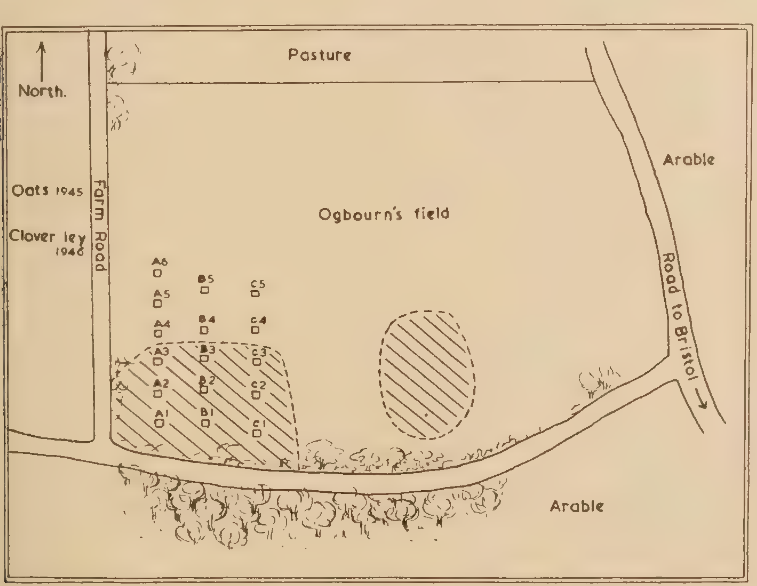


Fig. 3.—Sketch Map of Ogbourn's field showing damaged patches (cross hatched) and position of stations.

In the south-west corner of the field an area of approximately 3 acres was infested and further east, separated by a hollow in the field, a smaller area approximately 2·5 acres in extent (fig. 3). Only occasional larvae were found in the ground between these two areas and in the rest of the field.

The first of these two areas was selected for study and, in the spring of 1945, 16 stations were marked out in the positions shown in fig. 3. The three rows of stations commencing from the south-west corner of the field were denoted A, B and C, and the stations in each row were numbered in order from the south side of the field. The distance between the stations in each row was 15 yds and the distance between the rows approximately 30 yds.

Each station consisted of an area 5 × 5 yds. square. It was estimated that this area was sufficiently small for the population of *P. horticola* to be relatively homogeneous and sufficiently large to allow for samples to be taken at intervals over a period of three or four years. The corners of each station were permanently marked with stakes driven to within an inch of the ground.

As the third-instar larvae moved deeper into the soil in winter and some were to be found in the limestone brash, two types of soil samples were taken from each station. For eggs and young larvae each sample consisted of a cylinder of soil 2·5 ins. diameter and as deep as the soil would permit. These samples which were taken with a steel sampling tool could be removed rapidly and without crushing. As most of the eggs were laid in the top 3 ins. and the larvae fed in the surface layer, the risk of missing any when sampling was negligible. When sampling during the autumn and winter for third-instar larvae, blocks of soil 4 ins. square were excavated to just below the top of the brash. Each block was cut out with a flat trowel and took from ten to 15 minutes to excavate. There was some risk of crushing the larvae during the excavation but as they were then fully grown they could be recovered from the soil without difficulty.

The position of the samples removed from each station was randomised in the following way. Each station was regarded as comprising 144 squares 15 ins. × 15 ins. numbered at random from 1 to 144. The distribution of the numbers was recorded on a chart. The first sample was taken from the centre of the square numbered 1, the second from the square numbered 2 and so on. This ensured a random sampling on each occasion and that no two samples were nearer than 15 ins. To facilitate sampling, a rope net 5 yds. square with 15-in. mesh was made. Before each sampling, a piece of tape was tied to the top strand of each square from which samples were to be taken. The net was pegged down over each station in turn and a sample removed from the appropriate squares. The use of the net greatly increased the speed and accuracy with which the samples could be taken.

The holes made by the removal of the samples were immediately filled with soil taken from mole hills or from the side of the field.

In addition to the station samples, a number of special sq. ft. samples were taken to study the micro-distribution of the eggs and third-instar larvae. These samples were taken by dividing a sq. ft. of turf into 16 blocks each 3 ins. square and excavating each block in turn to as great a depth as was possible.

Examination of Samples.

The 2·5-in. cores.—The eggs and larvae were extracted from these samples by means of the Salt-Hollick extraction method. This method has been fully described (Salt and Hollick, 1944), so details will not be given here save to mention one modification necessary for extracting eggs.

In the final stage of the extraction process Salt and Hollick separated the insects from the vegetation by shaking in a mixture of xylol and water. As the cuticle of most insects is wetted by xylol while the vegetation is not, the insects accumulated

TABLE IV.

Effect of different foods on fecundity. Number of eggs produced by individual weighed females.

No Food							Bracken							Blackberry						
	Eggs laid by							Eggs laid by							Eggs laid by					
Wt. of ♀ (mg.)	June 7th	June 15th	July 7th	Eggs laid	Residual	Total	Wt. of ♀ (mg.)	June 7th	June 15th	July 7th	Eggs laid	Residual	Total	Wt. of ♀ (mg.)	June 7th	June 15th	July 7th	Eggs laid	Residual	Total
53	–	–	–	–	2	2	45	1	–	–	1	5	6	51	–	3	–	3	–	3
55	–	2 (1)	–	2	3	5	57	–	–	–	–	8	8	57	–	–	–	–	7	7
63	–	5	–	5	–	5	57	9	–	–	9	–	9	57	–	–	–	–	8	8
66	6 (2)	–	–	6	1	7	69	4	5	–	9	–	9	66	–	9	–	9	1	10
74	–	10	–	10	–	10	69	–	14 (1)	–	14	–	14	71	–	12 (1)	–	12	–	12
76	–	14 (2)	–	14	1	15	77	–	11	–	11	–	11	77	–	5	5	10	–	10
79	–	1	1	15	–	15	77	–	10	–	10	–	10	77	4 (1)	–	11 (2)	15	–	15
80	4	6 (1)	–	10	–	10	84	–	–	–	–	20	20	83	–	14 (1)	–	14	–	14
91	15	1	–	16	–	16	85	–	–	–	–	15	15	87	–	21	–	21	–	21
92	–	21	–	21	1	22	95	–	19 (3)	–	19	–	19	95	–	14 (1)	–	14	2	16
94	5 (1)	20	–	25	–	25	99	–	16	–	16	–	16	100	–	19 (2)	–	19	–	19
105	6 (1)	13	–	19	–	19	127	15 (1)	14	–	29	–	29	119	–	14 (1)	15 (2)	29	1	30
Total	36	106	1	143	8	151		29	89	–	118	48	166		4	111	31	146	19	165
Infertile Eggs	4	4	–					1	4	–					1	6	4			
Mean				11·9		12·6					9·8		13·8					12·2		13·8

Burnet							Grass						
	Eggs laid by							Eggs laid by					
Wt. of ♀ (mg.)	June 7th	June 15th	July 7th	Eggs laid	Residual	Total	Wt. of ♀ (mg.)	June 7th	June 15th	July 7th	Eggs laid	Residual	Total
48	–	8	–	8	–	8	49	–	–	–	–	6	6
57	–	5	–	5	–	5	56	–	–	–	–	8	8
61	11 (1)	6	–	17	–	17	61	9(1)	1	–	10	–	10
65	5	5	2	12	–	12	66	1(1)	7(1)	1	9	–	9
70	5	18	–	23	–	23	71	3	11	–	14	–	14
76	14	7	–	21	–	21	76	1	12(2)	–	13	–	13
81	11 (1)	8	–	19	–	19	77	12	–	–	12	5	17
80	–	12 (1)	2	14	–	14	84	12(1)	1	–	13	–	13
90	–	8	12 (2)	20	–	20	87	6	1	–	7	9	61
97	8 (1)	8	5	21	–	21	95	21(2)	–	–	21	–	21
104	14 (1)	10	1	25	–	25	100	–	16	–	16	1	17
110	4	18	1 (1)	23	–	23	115	18	4	–	22	–	22
Total	72	113	23	208	–	208		83	53	1	137	29	166
Infertile Eggs	4	1	3					5	3	–			
Mean				17·3		17·3					11·4		13·8

Total eggs laid = 752. Figures in brackets denote numbers of eggs laid that were infertile.

in the xylol layer and the vegetation remained in the water below. The eggs of *P. horticola* are peculiar in that the chorion is wetted by water so that they remain below the interface and there is a risk that they will sink and be lost. To avoid this risk, the xylol extraction was made over concentrated magnesium sulphate. The mixture of vegetation and insect material was washed into a beaker containing magnesium sulphate solution of density 1·18. A layer of xylol was run onto the surface and the interface agitated briskly with a pair of forceps. All the chitinous material came up into the xylol layer and could be collected in the usual way. The eggs which remained below the interface floating on the magnesium sulphate could be readily distinguished by their shape and colour when the surface was examined under a low-power binocular.

The 4-in. block samples.—By the time the larvae of *P. horticola* have reached the third instar they are of sufficient size to be extracted without loss by hand sorting the samples. As the soil from chafer infested areas is invariably light, it is particularly suited to examination by this method for the soil crumbs readily break down under pressure. The 4-in. block samples were therefore examined by this means. Each sample was spread on a tray, the soil broken down with the fingers and carefully examined, and the turf teased apart.

The sq. ft. samples.—The 3-in. blocks from the sq. ft. samples were examined by the Salt-Hollick extraction method for eggs in summer and by hand sorting for larvae in autumn and winter.

The data obtained from the examination of the sq. ft. samples (Table V), and station samples (Table VI), form the basis for the analysis of spatial distribution and seasonal changes in population.

Spatial Distribution of the Eggs.

Horizontal distribution.

Table VI shows that the horizontal distribution of the eggs as measured by the numbers recovered from the station samples in 1946 and 1947 varied greatly. If the eggs were distributed at random the numbers found in the samples should follow a Poisson distribution for which the variance is equal to the mean. The extent to which the distribution differs from random may be measured by Fisher's "coefficient of dispersion" given by the formula :

$$C = \frac{(x-\bar{x})^2}{\bar{x}(n-1)} \quad i.e., \quad \frac{\text{Variance}}{\text{Mean}}$$

which leads to unity for a population distributed at random, less than unity for an over-dispersed (*i.e.*, evenly distributed) population and more than unity for an under-dispersed (*i.e.*, aggregated population). The significance of the divergence from unity can be tested by the formula $2\sqrt{\frac{2n}{(n-1)^2}}$. A value of "C" lying outside the limits of $1 \pm 2\sqrt{\frac{2n}{(n-1)^2}}$ indicates that the population is significantly aggregated or over dispersed.

For the eggs recovered from the samples in June, 1945 and July, 1946 the coefficients of dispersion are 38·57 and 13·88 both of which are significant of aggregation. Turning to the sq. ft. samples (Table V), the coefficients of dispersion for the first two are 14·01 and 4·5 again significant of aggregation. (Limits 1 ± ·75 for 16 samples and 1 ± ·26 for 120 samples.) The third and fourth sq. ft. do not yield any information, though the recovery from 4 sq. ft. of. 55, 32, 7 and 0 eggs is itself indicative of aggregation.

Clearly the eggs are laid in groups and their grouping suggests that the adults select particular oviposition sites, for in many cases the number of eggs found in individual 2·5-in. core samples was equal to the average number of eggs laid by the female.

The data are too variable to permit a reliable estimate to be made of the difference between the numbers of eggs laid at the individual stations.

Vertical distribution.

The samples taken on 12th June 1945 were examined in two parts, an upper portion from 0 to 3 ins. deep and a lower portion comprising the remainder of the sample. Of the 103 eggs found in these samples, 84 were found in the upper portions and 19 in the lower portions, showing that the majority of the eggs are laid in the top 3 ins. of soil. As the soil over much of the field is only 3–4 ins. deep, it may be that in fields where the soil was deeper a greater proportion would be laid below 3 ins.

TABLE V.

Horizontal micro-distribution of eggs and third-instar larvae in sq. ft. samples.
Ogbourn's field.

Distribution of eggs in 3-in. squares.

Sq. ft. 1—4.7.1946

0	29	0	3	
0	17	0	6	
0	0	0	0	T=55
0	0	0	0	C=14·01

Sq. ft. 3—11.7.1946

0	0	0	0	
0	0	0	0	
7	0	0	0	T=7
0	0	0	0	

Sq. ft. 2—6.7.1946

6	3	9	0	
2	0	0	0	
0	0	8	0	T=32
0	4	0	0	C=4·5

Sq. ft. 4—11.7.1946

No eggs found

Distribution of larvae in 3-in. squares.

Sq. ft. 1—19.10.1944

5	6	1	3	
1	1	3	1	
4	5	2	3	T=43
2	1	0	5	C=1·25

Sq. ft. 4—10.11.1944

2	4	2	0	
4	4	0	1	
0	3	5	1	T=31
0	3	1	1	C=1·25

Sq. ft. 2—28.10.1944

9	3	1	2	
4	4	0	5	
2	3	3	1	T=48
3	1	2	5	C=1·33

Sq. ft. 5—6.12.1944

2	0	0	2	
2	2	1	1	
0	1	1	5	T=24
0	3	3	1	C=0·77

Sq. ft. 3—10.11.1944

2	1	1	1	
1	2	1	1	
1	1	5	2	T=27
1	3	3	1	C=1·55

Sq. ft. 6—17.3.1945

2	1	1	1	
4	2	4	2	
3	1	3	2	T=40
2	6	3	3	C=0·75

T=total C=coefficient of dispersion.

Spatial Distribution of the Larvae.

At the moment of hatching the distribution of the larvae is of course identical with that of the eggs. As the larvae move from the oviposition sites and as some of them die, their spatial distribution changes and becomes more and more dependent upon the effect of factors which are quite independent of those which determined the distribution of the eggs. The spatial distribution of the larvae, therefore, is dynamic and intimately related with seasonal movement and changes of population.

Horizontal distribution.

The coefficients of dispersion for the station samples taken on 28th July 1945 and 15th August, 1946 are 1·05 (16 samples), and 1·24 (120 samples), neither of which differ significantly from unity. The larvae are evidently distributed at random.

For the samples taken in January 1946 and November 1946 the coefficients of dispersion are 2·13 and 1·45, both of which are significant of grouping.

None of the coefficients of dispersion for the sq. ft. samples taken during the winter of 1944 differ significantly from unity.

The seasonal changes in the distribution of the larvae may be attributed to two factors, movement and mortality. The larvae gradually disperse from the oviposition sites and some of them die so that by August in each year their distribution is random.

The aggregation which occurs in the autumn may represent regrouping at feeding sites. If so, the evidence of the sq. ft. samples suggests that the feeding sites

TABLE VI.

Numbers of eggs or larvae from station samples, Ogbourn's field.

Station	1945				1946				1947			
	12th June	7th July	28th July	Jan. 1946	12th July	15th Aug.	15th Nov.	April 1947	10th July	15th Aug.	15th Nov.	April 1948
A1	*10*	*5*	*5*	0000 *0*	0000 0007 *7*	0000 0001 *1*	0000 1000 *1*	0000 0000 *0*	0000 0200 *2*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*
A2	*3*	*1*	*4*	0021 *3*								
A3	*16*	*10*	*4*	2141 *8*	0000 0032 *5*	0001 0110 *3*	1001 0000 *2*	0010 0001 *2*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*
A4	*2*	*0*	*7*	4112 *8*	0000 0000 *0*	1010 0211 *6*	0000 4011 *6*	0000 0100 *1*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*
A5	*0*	*1*	*5*	2321 *8*	0,14,00 0,21,01 *36*	0200 2532 *14*	1231 0141 *13*	0000 1102 *4*	0000 0000 *0*	1100 0100 *3*	0100 1021 *5*	0000 0000 *0*
A6	*11*	*0*	*0*	3043 *10*	4000 11,000 *15*	1101 0310 *7*	2103 0212 *11*	0011 1020 *5*	0000 0000 *0*	0000 0000 *0*	0000 1000 *1*	0000 0000 *0*
B1	*6*	*4*	*1*	2000 *2*	0000 0000 *0*	0000 0001 *1*	1000 0010 *2*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*
B2	*15*	*5*	*6*	2323 *10*	7000 0000 *7*	0120 0000 *3*	0000 0100 *1*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*
B3	*13*	*10*	*6*	4021 *7*	0032,3 0000 *35*	0000 1111 *4*	1000 0110 *3*	0011 0000 *2*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*
B4	*0*	*0*	*3*	1332 *9*	0000 0000 *0*	0120 1010 *5*	3000 3000 *6*	0110 0010 *3*	0100 0000 *1*	0100 0000 *1*	0000 1010 *2*	0000 0000 *0*
B5	*1*	*0*	*6*	2241 *9*	12,000 0000 *12*	0001 0110 *3*	0110 2100 *5*	0100 0000 *1*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*	0001 0000 *1*
C1	*26*	*4*	*2*	1054 *10*	0,12,00 0000 *12*	1000 1201 *5*	0000 0001 *1*	0000 0000 *0*	0000 0200 *2*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*
C2	*0*	*4*	*7*	4211 *8*	0020 0,19,02 *23*	0000 1100 *2*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*	0000 0000 *0*
C3	*0*	*7*	*5*	1237 *13*	0000 1205 *8*	1011 0012 *6*	0000 0011 *2*	0000 1011 *3*	0000 0000 *0*	1000 0000 *1*	0000 0000 *0*	0000 0000 *0*
C4	*0*	*11*	*5*	5043 *12*	6009 0000 *15*	0220 1000 *5*	0201 0001 *4*	0000 0000 *0*	0000 0400 *4*	0000 0000 *0*	0000 0000 *0*	0001 0001 *2*
C5	*0*	*5*	*2*	3212 *8*	1200 0201 *6*	0000 1002 *3*	0000 1020 *3*	0010 0001 *2*	0000 0000 *0*	0000 0000 *0*	0100 0210 *4*	0000 0000 *0*
Total	103	67	68	125	179	68	60	23	9	5	12	3

NOTES: The samples taken on Jan. 1946; 15th Nov. 1946; April 1947; 15th Nov. 1947 and April 1948 were 4-in. blocks, the remainder 2·5-in. cores.

are larger than one sq. ft. because, within an area of a sq. ft., the larvae are still distributed at random.

Vertical distribution.

Initially the vertical distribution of the larvae is the same as that of the eggs. After hatching, the larvae begin feeding and throughout the summer they are found in the root zone, often so near the surface of the soil that they can be found by scratching the surface. With the onset of winter, feeding ceases, the gut is emptied and the larvae move deeper into the soil to hibernate. The accompanying table giving data from sq. ft. samples taken during the autumn of 1944 shows these two processes in operation.

Source	Date	Larvae			Larvae		
		0–3 ins.	Below 3 ins.	% below 3 ins.	Abd. full	Abd. empty	% Abd. empty
Sq. ft. 1 ...	19 Oct.	28	15	34·9	33	5	13·2
Transect ...	24 ,,	17	15	46·9	16	11	40·7
Sq. ft. 2 ...	28 ,,	9	22	71·0	28	3	9·7
Sq. ft. 3 ...	10 Nov.	6	42	87·5	23	23	50·0
Sq. ft. 4 ...	16 ,,	1	23	95·8	3	21	87·5
Sq. ft. 5 ...	6 Dec.	6	21	77·8	0	27	100·0

Seasonal Changes in Population.

The data from the station samples provide the basis for this analysis of the seasonal changes in population at Ogbourn's field. In studying those changes it would be desirable to compare them for individual stations and then seek to correlate any differences with environmental factors which might explain them, but because of the variability of the data, it is not possible to do this. It is, however, possible to divide the stations into two groups and then seek to relate their data to environmental differences.

When the stations were marked out in the spring of 1945 the first three in each row were within part of the field where the turf had been severely damaged by larvae in the previous autumn. The turf at the remaining stations had been unaffected. In the autumn of 1945 the turf at the first three stations in each row was again damaged while that at the remaining stations was not. The stations may, therefore, be divided into two groups, the damaged and the undamaged.

The seasonal changes in population have been analysed first of all for the whole area covered by the stations and secondly for the areas represented by the two groups of stations. As the data do not follow the normal distribution, the statistical analysis has been made on a logarithmic transformation. Estimates of the population have been made for each occasion on which samples were taken. These estimates are given in Table VII together with the 5 per cent. fiducial limits. As the area covered by the stations was relatively small the estimates were made in numbers of eggs or larvae per sq. yd. rather than in numbers per acre.

The estimates of the total population for all stations have been plotted in fig. 4 together with the 5 per cent fiducial limits and the regression line giving the estimated seasonal variations.

It is clear that, in addition to the expected fall in population within each year, there is a marked fall in population between the years. This annual fall may be conveniently represented by estimates of the population at two arbitrarily chosen times, namely the number of eggs each year and the number of larvae on 1st October each year.

TABLE VII.

Population estimates, Ogbourn's field.

Estimates of number of eggs or larvae/Sq. Yd. with fiducial limits.

Total population, all stations.

Date	Estimate	5 per cent. Limits
12.6.45 ...	370	144–781
5.7.45 ...	359	193–610
28.7.45 ...	486	316–721
Jan. 46 ...	133	88–197
12.7.46 ...	230	101–478
15.8.46 ...	125	90–171
Nov. 46 ...	28	20–38
Apr. 47 ...	11	4–19
10.7.47 ...	12	0–27
15.8.47 ...	7	0–17
Nov. 47 ...	4	1– 8

Damaged stations			Undamaged stations		
Date	Estimate	5 per cent. Limits	Date	Estimate	5 per cent. Limits
12.6.45 ...	760*	276–1819	12.6.45 ...	115*	0–473
5.7.45 ...	646*	314–1218	5.7.45 ...	137*	13–371
28.7.45 ...	531	306– 870	28.7.45 ...	432	223–762
Jan. 46 ...	105	59– 180	Jan. 46 ...	182	89–323
12.7.46 ...	261	87– 688	12.7.46 ...	190	48–581
15.8.46 ...	93*	39– 140	15.8.46 ...	180*	115–275
Nov. 46 ...	14**	7– 22	Nov. 46 ...	63**	42–93
Apr. 47 ...	6	0– 15	Apr. 47 ...	18	7–37
10.7.47 ...	11	0– 33	10.7.47 ...	13	0–39
15.8.47 ...	3	0– 16	15.8.47 ...	12	0–29
Nov. 47 ...	0*	0– 5	Nov. 47 ...	11*	4–23

* or ** indicates significant or highly significant difference between pairs of estimates.

The estimates, derived from those of seasonal variation (fig. 4), are as follows :—Estimated number of eggs per square yard in 1945, 1946 and 1947, 436, 181 and 11 respectively ; estimated number of larvae per square yard on 1st October in 1945, 1946 and 1947, 250, 79 and 7 respectively.

Clearly at Ogbourn's field the infestation reached its peak at or before the time the investigations were begun and was steadily declining throughout the investigation.

Seasonal Changes within the damaged and undamaged Areas.

The fall in population within each year and from year to year is again visible from the estimates of the population of the damaged and undamaged areas (Table VII). The 5 per cent. fiducial limits enable a comparison to be made between the populations of the two areas on each occasion they were sampled. Estimates which differ significantly have been marked with an asterisk.

In June, 1945, there were significantly more eggs found at the damaged stations than at the undamaged. This difference was reflected in the numbers of larvae found on 5th July 1945. On 28th July and in January, 1946 there was no evidence of a significant difference.

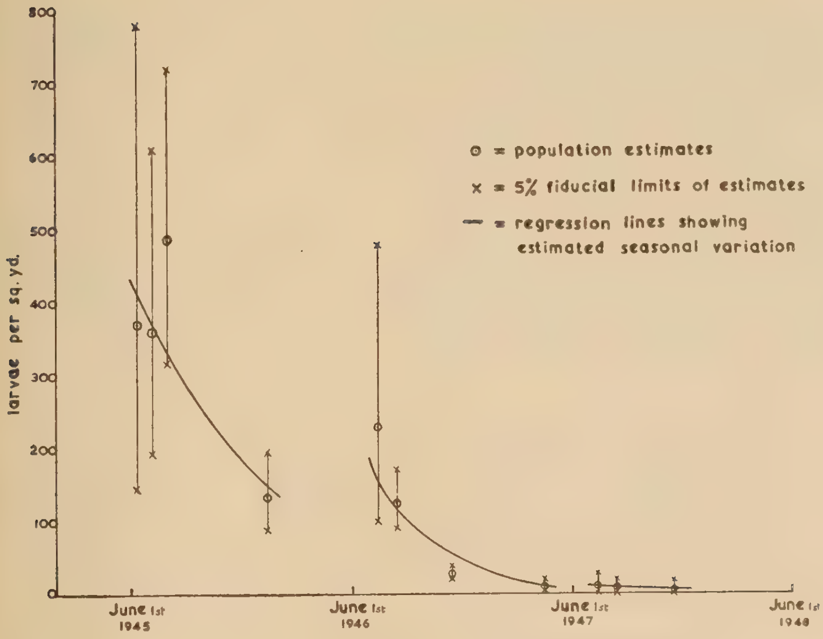


Fig. 4.—Population estimates Ogbourn's field 1945–47.

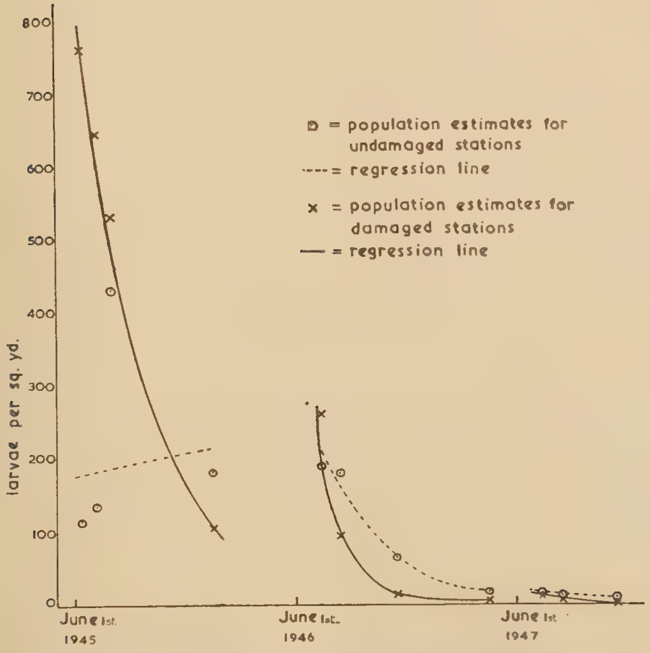


Fig. 5.—Population estimates Ogbourn's field, 1945–47.
Damaged and undamaged stations.

In July, 1946, a year in which development was retarded by the colder conditions, there was no evidence of a significant difference in the numbers of eggs within the two areas. By mid August, however, there were significantly more larvae at the undamaged stations and the same was true in November, 1946, the difference then being significant at the 1 per cent. level.

By 1947 the population in each area had fallen to a very low level but even so, in December there were significantly more larvae at the undamaged stations.

Here then is evidence of a change in degree of infestation between the two areas. At the beginning of the investigations the damaged stations were more heavily infested. The degree of infestation had changed over by the autumn of 1945 and from then onwards, whenever differences were significant, more eggs or larvae were found at the undamaged stations. This change is shown in fig. 5 where the estimated populations of the two areas have been plotted together with their regression lines.

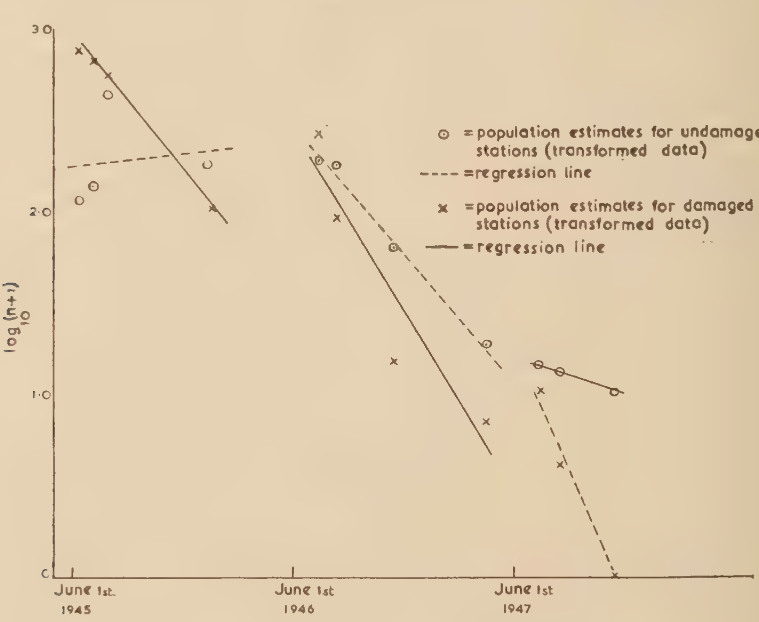


Fig. 6.—Mortality rate Ogbourn's field, 1945–47. Damaged and undamaged stations.

Turning now to the mortality of the larvae, the mortality rate within the two areas has been estimated based on the decline in population within the season, and is shown for the transformed data in fig. 6, together with the regression lines. The mortality rate each year was greater at the damaged stations. The difference was significant in 1947 only, but is confirmed by the other two years.

Analysis has shown that three processes were in progress during the course of the investigation.

(1) A general decline in infestation over the whole area.

(2) A relative decrease in infestation at the damaged stations and a relative increase at the undamaged stations.

(3) A higher mortality rate at the damaged than at the undamaged stations.

Our purpose now is to look for the factors responsible for these processes and those about which there is information will be discussed in turn.

Increased dispersal of Beetles.

Annual changes in population are dependent upon the extent of the seasonal fall in population and the fate of the adults. The estimates for Ogbourn's field suggest that both of these factors were involved in the annual fall in population. Each female lays an average of 14 eggs and it has been observed in rearing beetles in the insectary that males and females emerge in approximately equal numbers. If the population is not to decline, the numbers of beetles emerging in the spring must therefore be not less than one-seventh of the number of eggs laid the previous year. In 1946, the population fell to less than one-seventh of its original level and in both 1946 and 1947 the number of eggs laid was less than would be expected from the number of larvae surviving in the spring. It has been mentioned (page 609), that the beetles became more widely dispersed in 1946 than in 1945 and that they were found up to 50 yards into the adjoining field which was then in a clover ley. This increased dispersal of the beetles would result in a lower infestation in the area covered by the stations.

Vegetation.

Walton (1935) found that the beetles preferred a meadow to an adjoining pasture which was closely grazed and that, subsequently, more larvae were found in the meadow than in the pasture. Observations on some experimental plots in Ogbourn's field showed that a greater number of adults were active on unscythed manured plots than on similar plots which had been cut with a scythe. This evident preference for dense vegetation may account for the change in degree of infestation within the two areas, because the vegetation was less dense at the damaged stations (p. 636).

Consolidation.

It is characteristic of areas severely infested by *P. horticola* that the turf becomes soft and spongy. In the spring of 1946, some preliminary estimates of the firmness of the turf were made with an apparatus which measured the force required to tear the turf. The apparatus was kindly loaned on this occasion by the Air Ministry. Estimates made at eight points near the damaged stations showed that the average force required to tear the turf was 9·75 lbs. with a range from 4·5 to 13·5 lbs. Estimates made at twelve points near the undamaged stations showed that the average force required to tear the turf was 28 lbs. with a range from 21 lbs. to 36 lbs. Clearly the turf was firmer at the undamaged stations.

It is generally affirmed that chafer beetles prefer soft ground for oviposition. Gray described how he watched females trying without success to burrow into soil which was baked hard by the sun. On the other hand, in a preliminary trial in Dorset, consolidation by a farm roller did not cause any decrease in infestation. The evidence is conflicting, but at Ogbourn's field any preference which the beetles showed for soft ground was insufficient to prevent the change in degree of infestation.

Soil depth.

The soil depth at each station was estimated by making borings with a probe. These estimates are given in the composite Table VIII. In general, the soil was deeper at the undamaged stations. Soil depth is most likely to affect mortality during the winter, especially if there are severe frosts. During the exceptionally severe winter of 1946–1947, there was no evidence of a greater mortality at the damaged stations (fig. 6). Apparently the changes in population occurring within the field were independent of soil depth.

Organic carbon and Total nitrogen.

The percentage of organic carbon at each station was estimated by the Walkley and Black rapid titration method. Total nitrogen was estimated by the Kjeldahl method. The results are given in Table VIII. The two values for each station are closely correlated and give a C/N ratio of approximately ten for each station. In general, there was a higher percentage of organic carbon and total nitrogen at the damaged stations, with the exception of station C.1. It is difficult to see how organic carbon or total nitrogen themselves could affect oviposition or mortality unless the beetles prefer soil with a high content of organic matter. It may be that these values for carbon and nitrogen represent an effect rather than a cause, for by the destruction of the grasses greater quantities of organic matter would be returned to the soil at the damaged stations.

Soil moisture.

Estimates of the moisture content of the soil at each station were made in August, 1946. The results are given in Table VIII. The moisture content of the soil followed the percentage of organic carbon and total nitrogen very closely, no doubt because the organic matter increases the water retaining capacity of the soil.

When incubating the eggs from oviposition experiments, it was noticed that if the soil on which they were kept was rather moist the larva on hatching was unable to free itself from the egg shell, part of which remained sheathing the head capsule. In this way, considerable numbers of the larvae died just after hatching. Similar events occurred if the soil became dry. Evidently there was a limited range of moisture content essential for successful emergence.

The higher moisture content of the soil may be a factor involved in the greater mortality rate at the damaged stations. In 1945 and 1946 there was a more rapid fall in population at the damaged stations during the early stages in the life cycle (fig. 6). Gray recorded finding more larvae in dry friable soil than in moist soil.

Predators.

Birds are the most important predators, and are attracted to the loose turf from which they tear the larvae. The damage caused by the larvae is often greatly aggravated by the birds pulling out loose turf in their search for grubs. In the autumn of 1945, some rooks and starlings were active in the damaged areas and undoubtedly caused a reduction in the population. By the autumn of 1946 the larval population in the field was so low that no further damage to the turf occurred and in consequence birds were not active in the field.

There were, however, large numbers of moles occupying the hollow on the south side of the field between the two main areas infested by *P. horticola*. It was observed that, in the autumn, the moles migrated from the hollow and spread over the area covered by the stations but more especially those to the south east. No moles were trapped so no conclusive evidence of predation was obtained, but it is most probable that some of the larval mortality can be attributed to them.

Although many hundreds of larvae were examined during the investigations, none were found to be parasitised.

Although birds are recognised to be important predators no estimate of the extent of their predation has hitherto been made. Their activities are clearly dependent upon the degree of infestation and the extent to which the turf is damaged, for they are first attracted to infested fields in the early autumn when the turf is becoming loose and soft. When the turf remains unaffected, as it did at Ogbourn's field in 1946, they do not visit the fields.

A direct estimation of bird predation would be difficult to make, but an indirect assessment was made while sampling an infested field at Alton Pancras during the autumn of 1948. Nine contiguous plots, each 35 yds. × 25 yds. had been marked out in part of the field which had carried a fairly evenly distributed population of approximately 500,000 larvae per acre in the spring. These plots were sampled on 1st September, 1948, by taking ten pairs of 2·5-in. cores from each plot. The individual samples of each pair were one yard apart and the pairs were spaced evenly over the plots. When the samples were taken the larvae were just entering the third instar and no damage to the turf was visible.

In the succeeding weeks damaged patches rapidly appeared in the turf and flocks of rooks and starlings visited the field and considerably aggravated the damage while searching for the larvae. On 1st November, at the end of the feeding period when the larvae were beginning to hibernate, the plots were sampled again in the same manner.

On this second occasion the turf surrounding each pair of samples was inspected and graded "unaffected", "intermediate" or "damaged". By scoring +1 for each unaffected sampling point, 0 for each intermediate point and −1 for each damaged point an arbitrary estimate of the condition of the turf in each plot was obtained.

TABLE VIII.

Soil properties ; station data, Ogbourn's field.

Station	Organic carbon per cent.	Total nitrogen per cent.	Soil moisture per cent.	Soil depth (ins.)
A.1	9·02	·856	38·58	4·5
A.2	9·16	·894	41·30	5·0
A.3	8·22	·825	38·42	5·0
A.4	8·40	·823	38·40	5·5
A.5	5·94	·599	37·69	7·5
A.6	7·14	·695	38·36	8·0
B.1	7·61	·782	41·51	4·5
B.2	10·60	·993	46·40	4·5
B.3	7·97	·800	41·09	4·5
B.4	4·77	·556	34·95	8·0
B.5	4·80	·521	33·75	9·0
C.1	4·91	·531	38·18	4·5
C.2	9·66	·980	42·30	6·0
C.3	7·43	·757	39·47	5·5
C.4	5·37	·521	34·87	8·5
C.5	5·15	·546	35·06	8·5

The results of the sampling and grading of the plots are given in Table IX.

The number of larvae found in the samples taken on 1st September was 171 ; those taken on 1st November contained 84. In two months the larval population had been halved.

The fall in population for each plot is correlated with the extent of the damage to the turf, for there is a significant correlation between the fall in population and the plot score obtained from assessing the condition of the turf at each sampling point. ($r = -0{\cdot}767$. 5 per cent. value $= {\cdot}666$). This correlation is shown in fig. 7 where the change in population for each plot has been plotted against the score for the condition of the turf, and the regression line drawn in.

In order to estimate the effect of predation, it is necessary to compare the observed fall in population with the fall in population which would have occurred if the turf had remained unaffected by the larvae.

TABLE IX.

Relation between damage and fall in population; Alton Pancras, field plots. Numbers of larvae in ten pairs of 2½-in. core samples from each plot.

1st September 1948.

Plot 1			Plot 2			Plot 3			Plot 4			Plot 5		
0,0	0,0	1,0	1,2	2,1	1,1	0,1	1,3	0,2	0,0	1,1	1,1	3,1	0,1	0,0
0,1	0,1	1,3	1,0	2,4	2,3	1,2	0,1	0,1	0,0	0,2	0,0	0,1	1,0	0,3
1,2	0,0	3,1	0,0	0,0	0,1	0,1	0,3	0,0	3,2	0,0	0,1	0,0	0,0	1,3
	1,1	(16)		1,0	(22)		0,1	(17)		0,1	(13)		1,2	(17)

Plot 6			Plot 7			Plot 8			Plot 9		
2,0	0,1	0,1	0,1	1,3	0,2	1,4	1,2	2,0	1,3	0,1	1,0
0,1	3,1	0,0	1,4	4,0	1,1	1,1	1,0	1,2	1,1	0,0	3,2
4,0	0,0	0,4	1,1	0,0	1,2	1,0	0,0	1,0	1,1	0,0	1,1
	0,0	(17)		4,3	(30)		1,0	(19)		1,2	(20)

1st November 1948.

Plot 1			Plot 2			Plot 3			Plot 4			Plot 5		
D 0,1	U 0,2	U 0,1	U 0,0	D 1,3	U 0,0	U 0,1	D 0,0	0,0	0,0	U 0,0	2,0	1,0	U 0,0	U 1,0
1,2	U 0,0	U 1,2	U 1,0	U 1,0	1,1	0,0	0,0	U 0,0	D 0,0	U 0,0	0,1	0,0	0,0	1,1
2,2	U 0,0	0,1	1,2	0,0	U 0,0	U 1,0	D 0,2	D 1,0	U 2,0	1,0	2,1	U 1,0	1,0	0,2
+5	U 0,2	(17)	+4	0,0	(11)	+1	U 1,0	(6)	+3	U 1,0	(10)	+4	U 0,0	(8)

Plot 6			Plot 7			Plot 8			Plot 9		
D 0,0	U 2,0	0,0	0,0	D 1,0	D 0,1	D 0,0	D 2,2	D 1,0	0,3	U 0,0	0,1
D 0,2	U 0,0	U 1,1	D 0,0	D 1,1	0,1	0,0	0,0	0,0	D 0,0	U 0,0	D 0,1
D 0,0	0,0	0,0	U 0,0	D 0,1	0,0	U 0,0	0,0	1,0	U 0,0	U 0,1	0,1
0	1,1	(8)	—4	0,1	(7)	—2	1,2	(9)	+1	D 0,1	(8)

NOTE: Plot totals are given in brackets. Plot scores in November are given in the bottom left corner.
Damaged sampling points marked " D "; Unaffected marked " U "; Intermediate unmarked.

If the regression line were produced it would cut the x axis at approximately x=7 indicating that there would be no change in population on a plot with a score of +7. However, the regression between change in population and plot score cannot be entirely linear for the population cannot rise and so, while the regression line can approach the x axis, it may not cross it. Moreover, the method of scoring was arbitrary. It seems, however, that there would have been little change in population on a plot where the turf was unaffected (score +10). Obviously then the fall in population over this whole area at Alton Pancras can be attributed almost entirely to the fall in population in the damaged patches of turf and hence to the predatory activity of the birds.

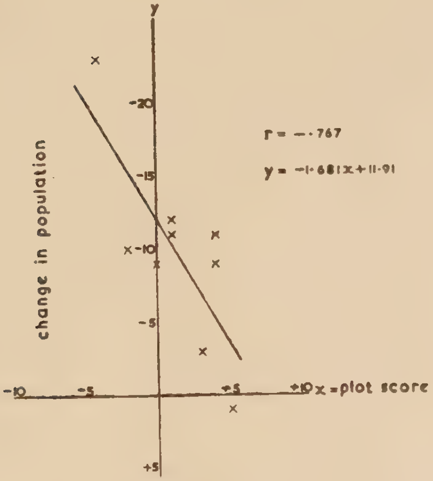


Fig. 7.—Relation between damage and fall in population.
Alton Pancras.

THE EFFECT OF INFESTATION UPON THE VEGETATION AND SOIL STRUCTURE.

It is generally affirmed that when grassland is attacked for several years by larvae of *P. horticola* the quality of the sward is reduced through a tendency for the fine-leaved grasses to be removed (Smith, 1931). Gray concluded that the deterioration of the sward was largely due to the colonisation of the damaged areas by weeds such as sorrel, plantain and yarrow. As no data are given, this conclusion was apparently based upon general observations made by inspection.

In order to obtain fuller information on the effect of infestation on the vegetation, the botanical composition of the sward was studied in relation to infestation at Ogbourn's field during 1945 and 1946. Supplementary data were obtained from Cadbury Camp in the autumn of 1946.

Ogbourn's field 1945–1946.

The two stations B5 and C5 were selected to represent an area in the field known to be infested but showing no damage to the turf. Stations B3 and C3 were selected to represent an infested area showing extensive damage (Pl. XXIII). Estimates of the botanical composition of the turf at these four stations were made in the winter of 1945, the spring of 1946 and the autumn of 1946.

In the winter of 1945 the turf at Stations B5 and C5 showed no signs of damage. At B3 and C3 where extensive damage had occurred, much of the ground was bare and the turf soft and spongy ; the larvae had just finished feeding and were hibernating in the deeper soil.

By the spring of 1946, five months after the end of the feeding period, the turf at Stations B3 and C3 was recovering from damage. Soon after the estimates were made, the field was vigorously harrowed and a dressing of 3 cwt. superphosphate, 1 cwt. sulphate of ammonia, and 1·5 cwt. muriate of potash per acre was applied. The infestation in 1946 was considerably less than in the previous year and the turf continued to recover during the summer ; the estimates made in the autumn of 1946 show this recovery from infestation aided by the dressing of fertiliser.

Estimates of the botanical composition of the turf were based upon the analysis of small quadrats as described by Blackman (1932), for grassland studies. A wire frame 6 ins. × 6 ins. was thrown down eight times at random at each station and the percentage area covered by each species estimated at each throw. The results of these estimates are given in Table X. Each figure is the average for the eight estimates with the exception of those for station C5 in the winter of 1945. These are based upon the analysis of a single sq. yd. divided into 36 × 6-in. squares.

For the most part identification of the grasses had to be based upon vegetative characters and it was difficult in the field to distinguish between the species of certain genera. Two species of *Agrostis, Avena, Festuca* and *Poa* were found; namely *Agrostis stolonifera* and *A. tenuis*, *Avena pratensis* and *A. pubescens*, *Festuca ovina* and *F. rubra*, and *Poa pratensis* and *P. trivialis*. For convenience in making the field estimations, no distinction was made between the two species of each genus. Similarly no distinction was made between the two species of clover, *Trifolium pratense* and *T. repens*, or between the three species of plantain, *Plantago lanceolata*, *P. media* and *P. major*. For convenience also, the daisy *Bellis perennis* and the ox-eye daisy *Chrysanthemum leucanthemum* were treated as a single species and are listed as *Bellis* sp.

In planning the survey it was expected that, in time, the turf at stations B5 and C5 would be damaged and that stations B3 and C3 would be recolonised. The changes in botanical composition associated with progressive damage and with recolonisation could then be studied simultaneously. Because of the decline in population in 1946, the changes due to progressive damage could not be studied in that way but had to be estimated by comparing the vegetation in the two areas represented by the stations.

The basic assumption in attributing differences between the two pairs of stations to the effect of infestation is that originally there was no difference in botanical composition between them. The data show that this assumption was not entirely valid. *Agrostis* was more abundant at B5 than C5, *Poa* was more abundant at C5 than B5; also plantains were more abundant at B5 and C5 than at B3 and C3, yet plantains damaged by chafer larvae have never been found even where damage has been most severe. Because of this, conclusions drawn from the survey must be somewhat tentative. Nevertheless, it is felt that they are of value because the nomadic disposition of *P. horticola* makes the opportunity for a thorough study of the problem in a straightforward manner very scarce.

No fewer than 41 species of flowering plants were identified during the study and it is possible that other species were present and included in " undetermined grasses ". Sixteen species were grasses, four were legumes and the remainder miscellaneous plants which may be regarded as weeds except for salad burnet *Poterium sanguisorba* which is a useful forage plant. The diversity of the vegetation, and in particular the low percentage of the area covered by legumes and high percentage by weeds, is indicative of the poor quality of the pasture and is characteristic of infested areas.

The general effect of infestation and the trend of changes during recovery may be seen in the summary of results in Table X. Initially the main difference between the two pairs of stations lay in the much greater percentage of bare ground at B3 and C3. This difference decreased during the survey due to the recolonisation of the damaged stations doubtless assisted by the fertiliser which presumably also caused the increase in the percentages of grasses at B5 and C5 by the autumn of 1946.

Throughout the survey the percentage of the area covered by weeds and legumes remained fairly constant with only small differences between the two pairs of stations except for the increase in clover at B3 in the autumn of 1946 and the fall in percentage covered by daisies and plantains at C3 in the spring of 1946. The difference between

TABLE X.
Botanical survey. Ogbourn's field.
Percentage area covered by each species at four stations.

	Winter 1945				Spring 1946				Autumn 1946			
	B5	C5	B3	C3	B5	C5	B3	C3	B5	C5	B3	C3
Dactylis glomerata ...	4·0	5·5	5·0	2·5	8·0	5·5	3·0	4·0	13·0	3·5	5·5	5·5
Agrostis sp.	10·0	4·5	·5	4·0	12·0	·5	·5	3·0	12·5	6·0		7·0
Lolium perenne ...	3·0	4·0	4·5	3·5	6·5	3·5	7·5	6·5	3·5	10·0	8·0	4·5
Poa sp.	13·0	17·0	6·0	1·0	11·0	26·5	7·0	7·0	8·5	15·5	18·0	8·0
Festuca sp.	14·5	5·5	< ·5	1·5	11·0	7·5	3·5	6·0	9·5	5·5	5·0	2·5
Holcus lanatus ...	5·5	2·0	< ·5			3·0	< ·5	·5	7·0	7·0		·5
Avena sp.	2·0	·5	4·0		2·5		6·0	5·5		3·5	4·5	4·5
Cynosurus cristatus ...	2·0	·5	< ·5	·5	< ·5	1·5	< ·5		3·0	1·5	6·0	1·5
Alopecurus pratensis		10·0										
Briza media	< ·5		< ·5	< ·5			3·0	2·0				3·0
Trisetum flavescens ...	1·5				1·0	< ·5		·5	< ·5	< ·5		
Koeleria cristata ...							3·0	< ·5				
Undetermined grasses	2·0		2·0	1·0	4·5	4·5	2·5	5·0	6·0	6·5	2·5	2·5
Total grasses ...	57·5	49·5	23·0	14·0	56·5	52·5	36·0	40·0	63·0	59·5	49·5	39·5
Trifolium sp. ...	1·0	1·5	·5	·5	1·0	1·5			2·0		12·0	·5
Lotus corniclatus ...	< ·5	3·0	2·5	3·0	< ·5	·5	1·0	< ·5	1·0	1·0	4·5	2·0
Medicago lupulina ...									< ·5	< ·5	< ·5	·5
Total Legumes ...	1·0	4·5	3·0	3·5	1·0	2·0	1·0	< ·5	3·0	1·0	16·5	3·0
Poterium sanguisorba	2·0	1·0	3·5	8·5			2·0	2·5	2·0	·5	7·0	4·0
Plantago sp.... ...	11·0	10·5	1·0	6·5	10·0	8·0	6·5	1·5	11·0	7·5	4·0	1·0
Bellis sp.	5·0	11·0	17·0	19·5	2·5	3·0	12·5	13·5	6·5	12·0	14·5	20·0
Hypochoeris radicata		1·0			1·0		·5		·5	1·0		·5
Leontodon hispidus...	1·5	1·5							1·0	5·5		·5
Taraxacum officinale	1·0					3·0		< ·5		< ·5		
Achillea millefolium...										·5	1·0	
Centaurea nigra ...			2·0	·5					·5		1·0	
Cerastium vulgatum...	1·0	·5		< ·5	·5				·5	1·0	·5	< ·5
Rumex acetosa ...	·5	·5				< ·5			1·0			
Luzula campestris ...	·5	·5		·5					< ·5			·5
Galium verum ...				·5						·5	1·0	·5
Primula veris ...			2·0							·5		< ·5
Orchis sp.						1·0						
Carex praecox ...	< ·5	< ·5	< ·5	1·5	< ·5		1·5	·5				2·5
Ajuja reptans... ...									< ·5			
Ranunculus bulbosus	< ·5	< ·5			2·5	1·5			1·0	< ·5	< ·5	
Umbelliferous seedlings					8·0	13·5	·5	2·5				
Moss	·5				1·0	1·0	< ·5			·5		
Total miscellaneous sp	23·0	26·5	25·5	37·5	25·5	31·0	23·5	20·5	24·0	29·5	29·0	29·5
Bare ground... ...	18·5	19·5	48·5	45·0	17·0	14·5	39·5	39·5	10·0	10·0	5·0	28·0

the stations and the changes occurring during the survey are largely confined to the grasses. Evidently, weeds are unaffected by infestation and there is no evidence of their colonisation at the expense of the grasses.

In considering the effect of infestation on individual species only those occurring regularly in appreciable quantities will be taken into account.

Dactylis glomerata.—With the exception of station B5, where there was a regular increase, *Dactylis glomerata* was found in approximately equal amounts at all four stations and therefore appears to be unaffected by chafer infestation. The increase at B5 is probably real, indicating a spread in that area.

Lolium perenne.—This was found in equal amounts at each station in the winter of 1945 and the amounts remained relatively constant during the survey. Its distribution closely resembled that of *Dactylis glomerata* and the evidence suggests that it too is unaffected by infestation.

Agrostis spp.—The distribution of *Agrostis tenuis* and *A. stolonifera* at the four stations was uneven but as the proportion at each station remained fairly constant it may be that *Agrostis* is unaffected by infestation.

Poa spp.—The large difference between the pairs of stations in the winter of 1945 and the subsequent steady increase at B3 and C3 suggests that *Poa* spp. are destroyed as a result of infestation but have considerable powers of regeneration, and under favourable conditions will recolonise the damaged area.

Festuca spp.—The distribution of *Festuca ovina* and *F. rubra* closely parallels that of *Poa* spp. suggesting that they too are destroyed by infestation but will regenerate under favourable conditions though to a less extent than *Poa* spp.

Legumes.—The leguminous species *Trifolium pratense, Trifolium repens, Lotus corniculatus* and *Medicago lupulina* were not abundant. The percentage at all four stations was approximately equal and remained constant with the exception of a sharp increase at B3 in the autumn of 1946. Apparently legumes were unaffected by the infestation.

Miscellaneous species.—At no time have plantains, daisies or ox-eye daisies been found to be damaged by chafer larvae, nor have the adults been observed feeding on them. It is maintained therefore that these weeds are unaffected by infestation, the significant point being that there was no evidence of their spread in the damaged area.

Poterium sanguisorba.—*P. sanguisorba* is a favoured food plant and there is evidence that it may increase the fecundity of the females of *P. horticola.* It is tempting therefore to suppose that while the percentage at each station remained constant its greater abundance at B3 and C3 accounts for the infestation being originally localised in that area.

Cadbury Camp, 1946.

Supplementary data on the effect of infestation on the vegetation were obtained from Cadbury Camp. The turf here, which was severely damaged in the autumn of 1946, is closely rabbit grazed and used only for rough grazing. A striking feature were the patches of the grass *Brachypodium pinnatum* which is resistant to rabbit grazing and apparently to chafer attack also because patches of it were still standing in areas devastated by chafer larvae (Pl. XXIII, fig. 2). Closer examination showed that there were the remains of other grasses killed by the chafer larvae within the patches of *B. pinnatum.*

Eight sq. ft. quadrats were examined in four pairs, the percentage area covered by each species being recorded for each sq ft. The first of each pair was taken from the centre of a severely damaged patch and the second from nearby ground showing no damage. The second sample of the fourth pair was exceptional as it was taken from the centre of a patch of *B. pinnatum* growing inside a severely damaged area.

The results of the survey are given in Table XI and three points of interest emerge.

(1) The vegetation was again characterised by its diversity. Much of the ground was covered by weeds, and the percentage covered by legumes was small. Apart from *B. pinnatum* five other grasses were identified, *Festuca rubra, Agrostis* sp., *Cynosurus cristatus, Lolium perenne* and *Avena pratensis. Festuca rubra* was the

TABLE XI.

Botanical survey. Cadbury Camp.

Percentage area of sq. ft. samples covered by each species.

	1st Pair		2nd Pair		3rd Pair		4th Pair	
Sq. ft.	1	2	3	4	5	6	7	8
Festuca rubra	6·5	36·0		24·5		37·0		1·0
Agrostis sp.	1·0	10·0		11·5		10·5		<·5
Lolium perenne	1·0	7·0		16·0		5·5		
Avena sp.				2·0		3·5		2·0
Cynosurus cristatus	1·5	2·5		1·0				
Brachypodium pinnatum ...						2·0		18·0
Unidentified	1·5	1·0						
Total grasses	11·5	56·5		55·0		58·5		21·0
Lotus corniculatus		5·0		·5		4·0		
Medicago lupulina	<·5	3·5		·5		·5		
Total Legumes	<·5	8·5		1·0		4·5		
Poterium sanguisorba ...	4·0	6·5	*	13·0	*	10·0	*	2·5
Plantago sp.	6·0	12·0	*	10·0	*			
Bellis sp.		6·0		1·0		1·0		
Leontodon hispidus	3·0	1·0						
Taraxacum officinale ...		1·0		<·5				
Senecio jacobaea		·5		<·5		<·5		
Hieracium pilosella	<·5	1·0		<·5		<·5		
Carex praecox	4·0	1·0	*	4·0		11·5		·5
Thymus serpillum		5·0						
Galium verum	1·5							
Cirsium sp.	2·0			11·0				
Ranunculus bulbosus ...	<·5	<·5		2·0		<·5		
Moss	·5	·5		<·5				
Total miscellaneous species	21·0	34·5	<5·0	43·5	<5·0	23·0	<5·0	3·0
Bare ground...	67·5	·5	<95·0	·5	<95·0	14·0	<95·0	76·0

*Indicates presence of this species.

First sample in each pair from a severely damaged patch, second from nearby ground showing no damage.

dominant species. *Lotus corniculatus* and *Medicago lupulina* were the only legumes found ; of the miscellaneous species, *Plantago lanceolata, Bellis perennis* and *Poterium sanguisorba* were dominant.

(2) *Brachypodium pinnatum* is resistant to chafer attack and will survive when other grasses are completely destroyed. This resistance is attributed to its tough rootstock which superficially resembles the creeping rootstock of bracken.

(3) Resistance of other grasses is purely relative, for under severe conditions they may be completely wiped out. It is noteworthy, however, that three species which proved relatively resistant at Ogbourn's field, namely *Dactylis glomerata, Poa pratensis* and *Poa trivialis* were not found at Cadbury Camp and the dominant species there, *Festuca rubra*, was destroyed at Ogbourn's field and regenerated rather slowly. This difference in the flora of the two areas may partly account for the severe damage at Cadbury Camp.

The Effect of Infestation upon Soil Structure.

It is characteristic of severe chafer damage that the turf becomes soft and spongy. The root system of the grasses may be almost completely destroyed and on examination the top 5 or 6 ins. of soil appears to consist of relatively small crumbs. In view of the importance of the soil tilth, and because it is known that grasses play an important part in the build up and maintenance of a stable crumb structure, it was decided to investigate what changes, if any, occurred in the soil structure owing to chafer infestation.

Method.

Methods of evaluating soil structure vary considerably and no standard technique appears to have been evolved. Of the many in use, those specifying the size distribution of the water-stable aggregates appear most common. In general, these methods consist of taking a sample of soil from the field, wetting it and sieving it, in water, on a bank of sieves of graded meshes to separate the aggregates into their respective size groups (Yoder, 1936 ; Tsyganov, 1935).

For the present work it was found necessary to modify the usual hand sieving method. A sample of soil from a damaged area could be sieved easily, but when a sample from an undamaged area was sieved the aggregates were held together by the ramifying grass roots. As it seemed inconsistent to regard particles held together by roots as single aggregates, some method of separating them from the roots had to be introduced. After several trials it was decided to prepare the sample for sieving by first washing it through a coarse sieve of approximately 8 mm. square mesh.

The method finally adopted was as follows. A block of soil about 6 ins. square and 5 ins. deep was cut from the area to be sampled, care being taken to reduce to a minimum any pressure on the block while cutting it. The samples were taken back to the laboratory in airtight tins and samples were prepared from them for sieving by carefully paring down the sides until a central portion weighing about 300 gms. was left. At the same time duplicate soil samples were taken for a moisture determination. After being weighed to the nearest gram, the prepared sample was placed on the 8 mm. sieve (fig. 8), which stood over a large container (B) full of water, and was wetted by spraying with an atomiser.

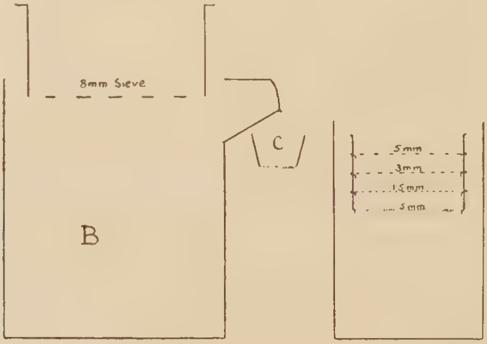


Fig. 8.—Diagram of apparatus for aggregate analysis.

After spraying, the sieve was lowered until it was about 1 in. below the water in the container and the sample was gently washed through the sieve. The grasses removed during the washing, and any pieces of vegetation caught in the overflow at C, were dried at 100°C. and weighed.

The sample was then carefully transferred on to a bank of graded sieves with meshes 5 mm., 3 mm., 1·5 mm. and 0·5 mm. diameter. The sieving was done by hand and was continued until the aggregates on the top sieve appeared to be of uniform size. The top sieve was then removed and the process continued until all the aggregates were separated into their respective size groups.

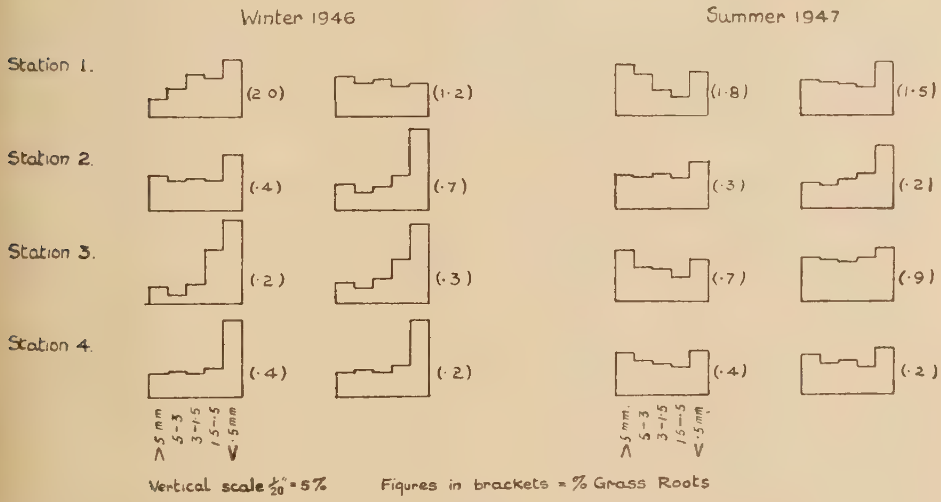
The aggregates from each sieve were dried at 100°C. and weighed. From the moisture estimation the weight of oven-dry soil in the original sample was calculated. The weight of soil passing the finest sieve was calculated by difference. The weight of aggregates of each size group was then expressed as a percentage of the oven-dry weight of the original sample.

Data were collected from Cadbury Camp 1946–47 and from Lytton Cheney 1947–48.

Cadbury Camp.

The severe damage at Cadbury Camp in the autumn of 1946 provided a good opportunity to make observations on the effect of infestation on soil structure. Four stations were chosen to represent a variety of conditions. Station 1 was in an area showing little damage ; station 2 was in the centre of a small damaged patch about 3 yds. in diameter ; station 3 was in an area devastated by the larvae, and station 4 was in the middle of a patch of *B. pinnatum* standing in a large damaged area. Two samples for aggregate analysis were taken from each of these stations in November 1946, when the larvae had just finished feeding, and again in the summer of 1947 when the turf showed some recovery.

The results of the analyses together with a summary of the vegetation are given in fig. 9.



	Station 1	Station 2	Station 3	Station 4
Percentage Grasses & Legumes	65·0	5·0	0	21·0
Weeds	34·5	5·0	5·0	3·0
Bare Ground	·5	90·0	95·0	76·0

Fig. 9.—Aggregate analysis, Cadbury Camp.

The soil structure at station 1 contrasts sharply with that at stations 2, 3 and 4. At station 1 there were approximately equal percentages of aggregates of each size group. At the other stations there was a marked increase in the percentage of aggregates less than ·5 mm. and a corresponding decrease in the larger aggregates.

As might be expected, the structure was poorest at station 3. It is noteworthy that although station 4 was covered by *B. pinnatum* the soil structure there was similar to that at station 2. Apparently the tough rhizomatous roots play little part in maintaining the soil structure.

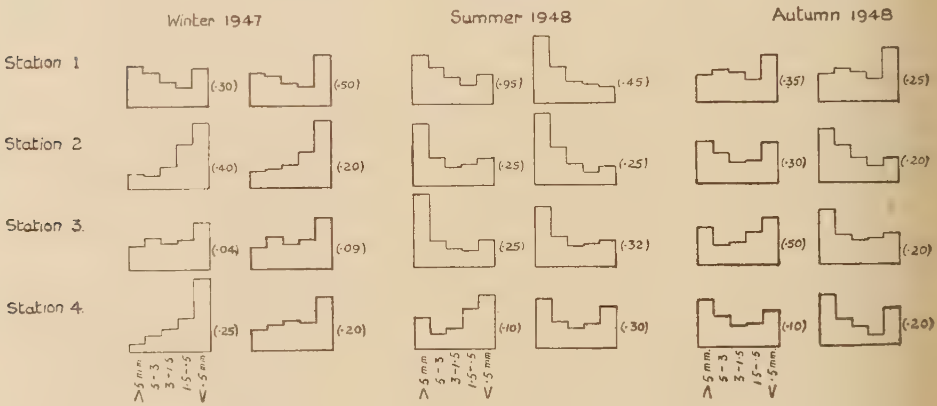
There appears to be a correlation between the soil structure and the amount of root material in the sample. The first sample at station 1 is however anomalous. Although there was a greater amount of root material in sample 1, the structure of sample 2 was better. This may be entirely due to a defect in the technique. There was a network of very fine roots in sample 1 making it difficult to wash the aggregates free, and hence subjecting them to greater breakdown in the washing process than in the other samples.

When resampled in the summer of 1947 the soil structure at the four stations was more nearly the same. The increase in percentage of the larger aggregates at the stations previously damaged was accompanied by an increase in the percentage of root material except for station 2 which showed an apparent decrease. This may have been due to sampling error owing to the relatively large variations which occur in a small damaged area. It is noteworthy, however, that station 2 showed the least recovery of structure.

Lytton Cheney.

At Lytton Cheney where observations were made in the winter of 1947 and the summer of 1948, an additional factor was introduced in that the field was stocked with cattle. Four stations were selected. Station 1 was apparently unaffected in 1947. The other three were damaged to about the same extent. All the stations, and in particular station 3, were subjected to consolidation by the cattle. Two samples for aggregate analysis were taken from each of the stations in the winter of 1947, and in the summer and autumn of 1948.

The results of the analyses are presented in fig. 10.



Vertical scale $\frac{1}{10}$" = 5%. Figures in brackets = % Grass Roots.

Fig. 10.—Aggregate analysis, Lytton Cheney.

The analyses made in the winter of 1947 show that at the damaged stations there was a greater percentage of aggregates less than ·5 mm. correlated with a lower percentage of grass roots than at station 1. That this correlation is not so marked as at Cadbury Camp may be due to the effect of consolidation, as station 3 where consolidation was greatest, had a better structure than stations 2 and 4 although the percentage of grass roots was less.

By the summer of 1948 there was a marked improvement in the soil structure especially at stations 2 and 3. There was a general increase in the amount of root material but it was not marked, and it is evident that consolidation by stock must be a factor both in maintaining the soil structure in the face of destruction of grass roots by the larvae and in its recovery after the larvae cease feeding.

The infestation in 1948 was slight, though some damage was to be seen in the autumn, especially near station 1. In consequence the soil structure changed little between summer and winter in 1948.

Discussion.

The soil tilth is a vague concept and the methods for measuring it are consequently approximate and empirical. The method used in the present work was necessarily simple and only large differences in soil structure could be demonstrated by it. In correlating changes in soil structure with the amount of root material, it must be emphasised that weight is a poor estimate of the effect that roots have on the soil structure. It seemed that the roots which were important were the relatively fine ones ; coarse roots such as those of *B. pinnatum* and weeds, though bulky, did not appear to influence the structure.

Nevertheless, it has been possible to demonstrate changes in soil structure attributable to the activities of chafer larvae and to point to two factors, grass roots and consolidation, which are important in maintaining and restoring soil structure in grassland infested by these larvae.

PRELIMINARY OBSERVATIONS ON CONTROL MEASURES.

Preliminary observations on three potential means of control, by cultivation, reseeding and application of insecticides will be briefly discussed here.

Cultivation.

It is well known that the population of macro-organisms in the soil may be greatly reduced by the violent change in the environment brought about by ploughing (Evans & Guild, 1948 ; Roebuck, 1924). Not only are the conditions less stable in arable land but the cultivations may damage the organisms, expose them to predators or the risk of desiccation. Cultivation of infested fields readily suggests itself as a possible means of control.

None of the fields which have been studied in detail has been ploughed and put to arable crops but several others have been examined after ploughing. In no case was any damage to the following crop observed and the farmers did not subsequently report finding larvae in the soil. Evidently ploughing will greatly reduce populations of *P. horticola*.

Larvae of *P. horticola*, however, do occur in cultivated land in sufficient numbers to be of economic importance, for in some forest nurseries on light soil seedlings may be seriously damaged. At Ampthill, in Hertfordshire, larvae of *P. horticola* were found in a nursery of coniferous seedlings which had been under cultivation for several years. No accurate estimate of the population could be made but it was certainly not great. It may be that in cultivated land, where there is a much lower plant population than in grassland, a relatively low larval population will cause extensive damage. This adaptation to the changed environment of a forest nursery needs further study.

Reseeding.

The poor quality of much of the land infested by *P. horticola* has already been emphasised. Improvement is frequently attempted by direct reseeding, more especially as it is possible to reseed land too hilly or too shallow for arable cropping.

Reseeding exposes the larvae to the effects of cultivation but adults from larvae which survive may oviposit in the new grass.

At the end of March 1948, a field at Barcombe Farm, Alton Pancras, which had carried a field experiment in 1947 (see below), was ploughed and reseeded direct to a cocksfoot ley. Prior to reseeding, the larval population of the area covered by the field experiment was estimated after examination of 128 × 2·5-in. core samples to be 240,000 per acre. In the autumn of 1948, two larvae were found in 40 × 2·5-in. core samples and the population estimated at 64,000 per acre. Unfortunately it was not possible to discover the extent to which the fall in population was due to the larvae being killed by the cultivation or to the new ley being unfavourable for oviposition.

Management of reseeded grassland may be important in restricting re-infestation. Walton (1935) found that adults preferred a meadow to an adjoining pasture which was closely grazed. Observations on experimental plots at Ogbourn's field confirmed this, for adults were more numerous on manured plots with dense herbage than on manured plots which were kept scythed. Grazing, whereby the grass is kept short and the turf well consolidated would appear to be preferable to cutting for hay.

Application of Insecticides.

In many areas where arable cropping or reseeding is impracticable, chemical control appears to offer the only solution. Previous attempts have been directed against the larvae, thus encountering the difficulties of exposing a soil insect in grassland to a lethal concentration of the insecticide. Control of the adults during the flight period appears to present fewer practical difficulties.

At the beginning of the flight period in 1947 preliminary experiments were carried out to see which of two insecticidal dusts, 5 per cent. DDT or 3·5 per cent. benzene hexachloride, was more toxic to the adults. Small patches of lawn were dusted with each insecticide at the rate of 70 lb. per acre. Beetles were caged on these patches for 20 mins. and then removed and kept separately until they died. Of 40 adults caged for 20 mins. on turf dusted with 3·5 per cent. benzene hexachloride, 36 were dead the following day and all were dead after three days. Of 40 similarly caged on turf dusted with DDT, none was dead the following day, five were dead after three days, 19 after four days and eight were still alive at the end of a week.

Small plots of approximately 20 sq. yds. were dusted with each insecticidal dust at the rate of 70 lb. per acre by means of a rotary blower during the flight period, when the beetles were active. Beetles were then immediately collected from the plots. Of 31 collected from plots dusted with 3·5 per cent. benzene hexachloride, 25 were dead the following day and four were moribund ; all were dead after two days. Of 32 collected from plots dusted with 5 per cent. DDT all were alive the following day though three were moribund ; four died after two days and nine more after four days. It was concluded that 3·5 per cent. benzene hexachloride dust was more toxic and acted more rapidly that 5 per cent. DDT dust. It was therefore used in subsequent field trials.

A field at Barcombe Farm, Alton Pancras, was sampled in April 1947 and part of it found to contain a fairly evenly distributed population of approximately 800,000 larvae per acre. Eight contiguous plots, each 21 yds. by 19 yds., were marked out in the centre of this area ; four were dusted with 3·5 per cent. benzene hexachloride dust at 70 lb. per acre and four were used as controls.

Biological studies (p. –), which had shown that males emerge before females and that a few days may elapse between mating and oviposition, provided a useful guide in timing the application of the insecticide. The first beetles were seen on 31st May and the plots were dusted on 6th June using a rotary duster. It was a calm day and there was little or no drift.

The following spring 16 × 2·5 inch core samples were taken from each plot and xamined for larvae. The results are given below. Two larvae were found in the 4 samples from the dusted plots and 22 in the 64 samples from the control plots.

Larvae found in 16 × 2·5 in. samples from each plot.

Control	Control	BHC	BHC
4	5	0	0
Control	BHC	BHC	Control
7	0	2	6

learly dusting had greatly reduced the population.

A similar experiment was carried out in 1948 with two rates of application of ust, 35 lb. and 70 lb. of 3·5 per cent. benzene hexachloride per acre. Nine plots ach 35 yds. × 25 yds. giving three replicates of each treatment and control were arked out in another field at Barcombe Farm which in March 1948 had a larval opulation of approximately 300,000 per acre. Dusting began on 1st June using a taniland dusting machine, but was not completed until 3rd June on account of most nfavourable weather with high winds and intermittent driving rain.

At the beginning of September, 20 × 2·5-in. core samples were taken from each lot and examined for chafer larvae. The results are given below. Analysis shows hat neither treatment had a significant effect.

Larvae found in 20 × 2·5-in. samples from each plot.

35 lb. BHC	70 lb. BHC	Control	35 lb. BHC	70 lb. BHC
17	13	17	22	16
	Control	35 lb. BHC	70 lb. BHC	Control
	20	19	30	17

iscussion.

These preliminary observations show that the three control measures merit fuller vestigation. Of the two indirect methods arable cropping is probably the more ficacious but reseeding is of wider application. Much of the land infested by *P. rticola* is suitable only for rough grazing and is of such poor quality that its improveent is needed irrespective of the efficacy of reseeding as a control measure. hemical control by means of 3·5 per cent. benzene hexachloride apparently depends r its success upon favourable weather at the time of application.

Some of the infested areas in the Lake District and Dorset Downs present practical fficulties in the application of control measures which appear insurmountable, for ltivation or reseeding is impossible and insecticides could only be applied effectively om the air. Yet these areas have an importance to the farmer quite out of prortion to their intrinsic value. At Lytton Cheney, *P. horticola* for the most part fests the higher slopes of the downs. In recent years, however, it has spread to ore valuable pastures on the lower slopes. Any control measures applied to these stures will be merely palliatives as long as the higher slopes remain infested.

GENERAL DISCUSSION.

This study of the ecology of *P. horticola* has shown clearly the interaction of the insect and its environment. An increase in the numbers of larvae produces changes in the environment and brings into operation a number of factors which directly or indirectly reduce the population and restore the balance. An interesting feature of the interaction of *P. horticola* and its environment is the violence of the changes and the extent of the fluctuations in population. It was seen that in some circumstances the turf was so damaged that over 95 per cent. of the ground was denuded of vegetation. Within two years the population of a field fell to less than 4 per cent. of its original level and within two months a population was halved by predators. This unstable equilibrium is in sharp contrast with that of some other soil insects such as wireworms, the population of which in natural grassland remains relatively stable.

The changes in population of *P. horticola* result chiefly from the extensive damage to the turf caused by the phytophagous larvae. In the damage it causes to grassland, *P. horticola* differs from most other phytophagous insects ; wireworms, for instance, are to be found in the soil in large numbers but cause no visible harm to the turf. The rapid growth and short feeding period of *P. horticola* may account for the difference. When the larva hatches from the egg in June or July, it is scarcely 2 mm. long but by the end of the feeding period in November it has increased to five or six times that length and perhaps one hundred times in weight. The wireworm on the other hand takes four years to reach maturity.

The instability of populations of *P. horticola* suggests an imperfect adaptation to the environment. Doubtless the irregular geographical distribution and the sporadic occurrence of outbreaks are partly due to the large fluctuations in local abundance which have been shown to occur from natural causes. The chafers may represent a stage in the adaptation to phytophagous life from the coprophagous habit of the related scarabs. In this connection it is of interest to note that two species of dung beetles, *Aphodius howitti* Hope and *A. tasmaniae* Hope, though showing a preference for dung, will attack grasses and have become serious pests of heavily stocked pastures in Australia (Evans, 1941).

Much of the work which has been described has been exploratory. Three main problems which would repay further study emerge from it. They are the physiology of reproduction in relation to food supply, the selection of oviposition sites and the factors affecting larval mortality. The first of these has been brought to a stage where it could well be studied in the laboratory. The second and third need further field study before they can be reduced to a series of precise physiological problems.

The study of the botanical composition of the turf at Ogbourn's field in addition to demonstrating the relative resistance of different plant species and revealing one factor which may affect the distribution of *P. horticola*, raises the problem of phytophagous insects in relation to botanical succession. The changes in soil structure which accompany infestation by *P. horticola* show that insects cannot be regarded as passive inhabitants of the soil for they may materially affect its physical condition.

Summary.

The garden chafer, *P. horticola* (L.) is widely distributed in England and Wales but is more abundant in the western half of the country. Its natural habitat is poor quality permanent grassland characterised by a diversity of species of flowering plants and a high proportion of weeds. The soil of the infested areas is invariably light, the land is usually hilly and the rainfall relatively high.

Adults emerge suddenly in May or June according to the weather. Males emerge some days before females. Daily activity increases during the flight period which lasts 3–4 weeks. Beetles only fly in warm sunny weather.

The females lay an average of 14 eggs each. Fecundity is correlated with weight and is related to the food supply of the larvae. In nature, the weight of the adults may be limited through the exhaustion of the food supply of the larvae. Females will mate and lay fertile eggs without feeding. In general, feeding by the adult does not affect fecundity but in one experiment beetles fed on salad burnet produced and laid more eggs than beetles fed on bracken, blackberry, grass or kept without food. Over 90 per cent. of the eggs are fertile ; fertility decreases with the age of the adult. The incubation period of the eggs is about 22 days in the laboratory but about 28 days in the field.

The spatial distribution and seasonal changes in population of *P. horticola* have been studied within an infested field on the Tickenham Ridge, Somerset. The eggs are laid in groups from which the larvae disperse. At the end of the feeding period the larvae may be distributed at random but there is some evidence that regrouping at feeding sites occurs. Most of the eggs are laid in the top 3 ins. of soil. The larvae feed in the root zone but go deeper in the soil in winter to hibernate. The population fell in successive years within the area studied. Initially there was a higher infestation in part of the field where the turf was damaged by the larvae but the relative intensity of infestation changed and subsequently more eggs and larvae were found in part of the field where the turf was undamaged. There was a higher mortality among larvae in the damaged part of the field. These population changes have been related to a number of environmental factors which may account for them, in particular to dispersal of the adults, density of the vegetation, consolidation, soil moisture and predators. In a field at Alton Pancras, Dorset, predation by birds reduced the larval population by 50 per cent. between 1st September and 1st November.

A study of the effect of infestation upon the vegetation showed that damage was largely due to the destruction of the grasses. Weeds were unaffected and there was no evidence of an increase of weed area by colonisation of bare patches resulting from damage. *Brachypodium pinnatum* resisted attack owing to its tough rootstock. *Dactylis glomerata* and *Lolium perenne* were relatively resistant, *Poa* spp. and *Festuca* spp. were damaged but regenerated when feeding stopped and the population declined. Salad burnet, a favoured food plant, was more abundant in damaged areas and its distribution may influence the distribution of *P. horticola.*

When infestation is severe and the grass roots are destroyed, the soil aggregates are broken down. The soil structure was restored when the grasses regenerated, and by consolidation.

Field populations were reduced by ploughing and by reseeding. Dusting with 3·5 per cent. benzene hexachloride at 70 lb. per acre during the flight period gave promising results when applied in favourable weather.

Acknowledgements.

I am pleased to acknowledge the advice and encouragement I received from Professor H. W. Miles while carrying out this work. I am also indebted to Mr. L. N. Staniland, A.R.C.S. and to Dr. C. B. Williams for permitting me to continue the work while in their Departments. The field work could not have been carried out without the goodwill of Mr. Ogbourn of Tickenham, Mr. Waterman of Alton Pancras and Colonel Wordsworth of Lytton Cheney who generously allowed me every facility for field work upon their farms. Several colleagues helped me in the field and I particularly wish to thank Mr. J. F. Southey, B.A., for assistance with the botanical survey of Ogbourn's field and Mrs. S. M. Wadsworth and Miss J. Freeland, B.Sc. For advice on statistical matters I am indebted to Mr. G. M. Jolly, B.Sc., and Miss E. P. Poulton, B.A.

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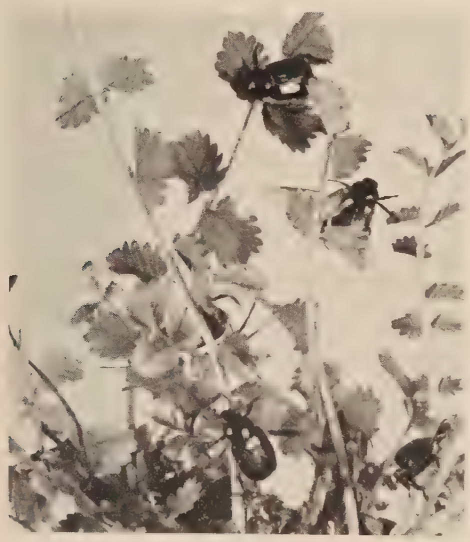

FIG. 1. *P. horticola* L. feeding on salad burnet. (Nat. size).

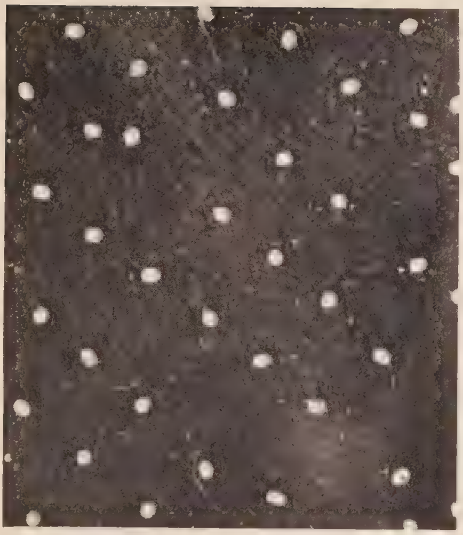

FIG. 2. Eggs of *P. horticola* L (x 1½).

FIG. 3. Larva of *P. horticola* L. feeding at grass roots.

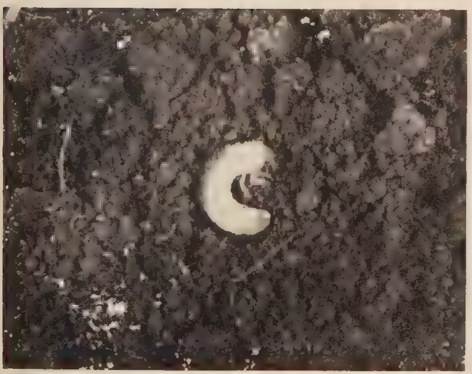

FIG. 4. Third instar larva of *P. horticola* L. (Nat. size).

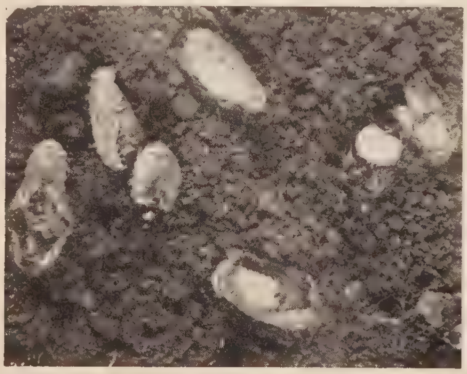

FIG. 5. Pupae of *P. horticola* L. with newly emerged adults. (Nat. size).

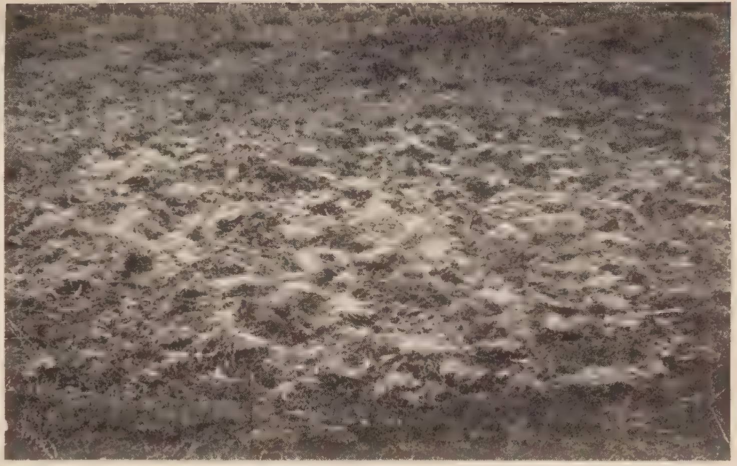

FIG. 1. Chafer damage developing in early autumn 1945.

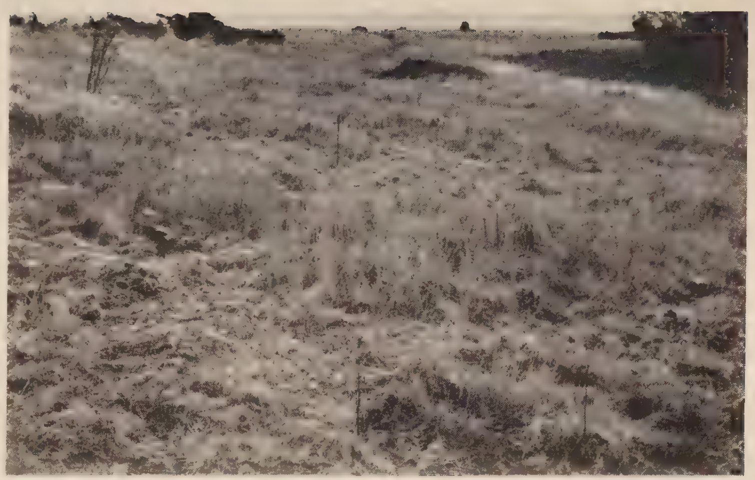

FIG. 2. Cadbury Camp autumn 1946 showing severely damaged area with *Brachypodium pinnatum* unaffected.

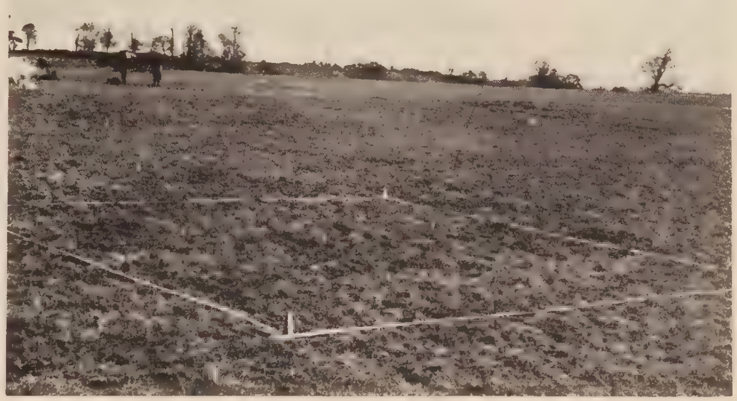

FIG. 3. Ogbourn's field. Station B5, autumn 1945.

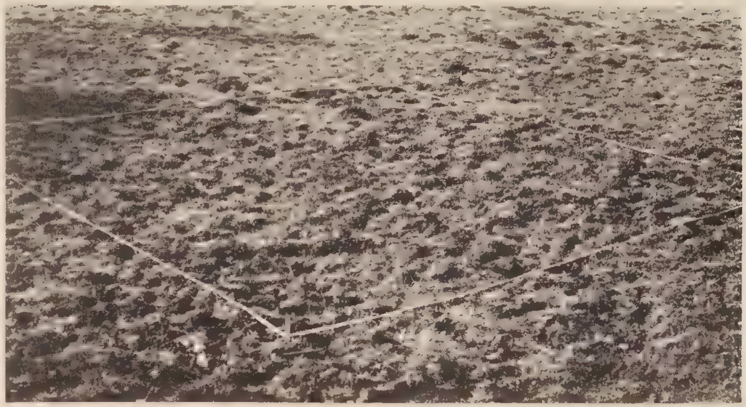

FIG. 4. Ogbourn's field. Station B3, autumn 1945.

A PRELIMINARY STATISTICAL STUDY OF *ANOPHELES GAMBIAE* GILES, BASED ON MAXILLARY INDICES.

By Robert W. H. Campbell,
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In 1946, a mosquito survey, covering Bathurst and part of the neighbouring mainland, was carried out by the writer. An important objective of the survey was to assess the public health status of the transocean aerodrome at Yundum, which lies inland some ten miles from the capital, and to put forward proposals for its protection from mosquito-borne disease. In connection with this work, random samples of adult mosquitos were systematically taken, not only from control houses on the airport itself, but also from a number of catching stations in the surrounding villages. The large amount of material, collected in this way, was first of all identified on gross morphological characteristics and later, dissected. In every case, the number of maxillary teeth was noted, with the intention at the time, of establishing a mean maxillary index for fresh-water breeding *A. gambiae* Giles, which later might serve,

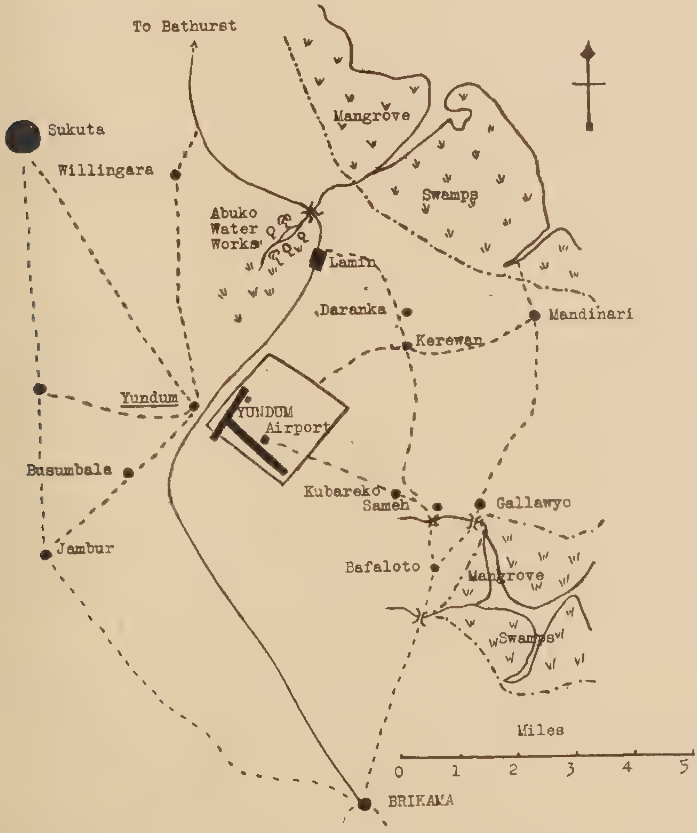
To Bathurst
Sukuta
Willingara
Mangrove
Swamps
Abuko
Water
Works
Lamin
Daranka
Mandinari
Kerewan
Yundum
YUNDUM
Airport
Busumbala
Kubareko
Sameh
Gallawyo
Jambur
Bafaloto
Mangrove
Swamps
Miles
0
1
2
3
4
5
BRIKAMA

Fig. 1.—Sketch map of Yundum and environs.

it was thought, as a basis for estimating the proportions of *A. gambiae* and *A. gambiae* var. *melas* Theo., in a mixed sample. It was not, however, until late 1947 and early 1948, that time could be found to subject the mass of data, which had been accumulated, to statistical analysis.

In selecting the data for this purpose, it was important to ensure that the samples analysed should contain no specimens of "*melas*". Reference to the map (fig. 1) shows that Yundum airport is situated about four to five miles from the nearest saline water and that Yundum village, where the material upon which this paper is based was collected, is nearly five miles from the nearest saline breeding grounds beyond the Abuko Water Works. It has been assumed that at this distance "*melas*" would be absent from adult collections or, at any rate, would not be present in large enough numbers to affect the analysis significantly.

Experience had suggested that an apparent difference existed between the powers of flight of certain adult fresh-water breeding "*gambiae*" for it had been found that the sterilisation of a zone varying from half to three-quarters of a mile was sufficient to reduce wet season catches in the airport terminal buildings by nearly 90 per cent. but, when this was increased in width to over a mile and a quarter, no significant reduction was found. It was noticed that in the dry season, when the nearest breeding place was at the Water Works, a catch nearly as great as in the wet season was obtained. It was known that a large number of small breeding places existed, during and for some time after the rains, in the area lying between the sterilised zone and the Water Works and there appeared to be no simple explanation why the adults bred from these pools did not reach the terminal buildings when those from the Water Works managed to do so in the dry season. To account for this, a working hypothesis was adopted. This was that adults hatched out in the larger permanent collections of water, where food might be expected to be more abundant, had better stamina and hence better powers of flight than those hatched from pools of casual water where the larval food supply was not likely to be so varied or abundant. Pursuing this line of thought, a number of mosquito-netting tents were set up over breeding places of both categories and the newly emerged adults were compared, to see if any significant difference in size or nutritional status could be discovered. At the same time, a study of the micro-flora of breeding places was commenced in order to ascertain if any obvious difference in larval food supply could be detected. No significant difference in size or nutritional status was discovered, but the possibility that some food factor existed, which might account for the apparently greater powers of flight of mosquitos bred in permanent water, could not be excluded. The maxillary indices of these samples, which had been noted as a matter of routine, were found, when analysed, to fall into two well-defined distributions, with a mean index of 13·107 for specimens collected over casual water and 15·33 for specimens collected over permanent water at Abuko. The modal indices for the two groups were 13 and 15·5 respectively. It had also been noted that the frequency histogram of the series collected from the catching stations departed from normality and this, in itself, had been regarded, in view of the relatively large size of the samples, as at least presumptive evidence that they were not drawn from a pure population.

The total daily collections from the catching stations were comprised of two fractions; one fraction collected in window-traps and the other collected by a spray-sheet catch, early each morning. Although the data from each fraction were recorded separately, it was not at this time considered necessary that they should be analysed separately. However, when this was done, it was found that the mean maxillary indices of the window-trap and spray-catch fractions were 12·91 and 14·74 respectively, showing a difference between the means of 1·83. These means do not diverge widely from those found in the breeding place samples and the statistical analysis showed that it was improbable that the two portions of the total house catches could have been drawn from a pure population by random sampling.

At this stage, the evidence strongly suggested the existence of two races within the species *A. gambiae*, apart altogether from the variety "*melas*", whose presence in the material analysed was considered to be excluded by the distance from saline water of the catching stations, and the fact that the first analyses had been made on adults bred in completely fresh-water sites. In addition, there appeared to be strong evidence in support of the theory that the variation in the two races was in some way linked with the type of breeding place. At the same time, although this view was later to be weakened by an alternative and simpler explanation, it was considered that the statistical analysis of the window-trap and spray-catch fractions had also established the existence of differences in behaviour between the two races. It was, therefore, decided to carry the analysis further and with the help of controlled experiments to attempt to elucidate the problem. So far, no opportunity has arisen to extend the scope of the experiments and it is only possible to give the results of the preliminary work in this paper.

Statistical analysis of the material collected.

The mathematical arguments advanced in this paper are based on the recognition that variations in a pure population (in this case the variation in the number of maxillary teeth) are distributed *normally* in accordance with a mathematical law which states that the logarithm of the frequency at any distance x from the centre of the distribution is less than the logarithm of the frequency at the centre by a quantity proportional to x^2. Any significant departure from normality, which may be shown by statistical analysis, is evidence of the improbability that the sample analysed is drawn from a pure population.

The data which has been analysed, is collected together in Table I below. The histograms in figs. 2, 3, 4, 5, and 6, are the frequency histograms of maxillary indices, calculated from the different samples referred to above. The histogram in fig. 7, however, is that of an artificial sample constructed by adding frequencies of identical indices from the samples collected over the two types of breeding place. This has been done to allow comparison with the mixed sample collected from window-traps and spray-catches in the control stations. All the material from control stations, used for analysis, has been taken from the village of Yundum (see map, fig. 1), which is not less than four miles from the nearest saline water. As mentioned before, it is assumed that this precaution ensures that the samples obtained were pure samples of fresh-water breeding *A. gambiae* or, at least, that the numbers of "*melas*", included in the catches, could not be sufficiently large to affect the validity of the statistical analysis. It should be mentioned that, throughout the period of the observations, no specimen of *A. gambiae* with four-banded maxillary palps was ever taken in this or the group of neighbouring villages lying to the west of the airfield. The emergent adults, captured over selected breeding sites, had all, of course, bred in either rain-water pools or the catchment area of the drinking-water reservoir at Abuko.

TABLE I.

Frequency distribution of maxillary indices.

A	B	C	D	E	F	G
10	0	0	0	1	0	1
10·5	1	1	0	3	0	3
11	2	2	0	1	0	1
11·5	2	1	1	7	0	7
12	24	19	5	37	0	37
12·5	28	18	10	35	2	37
13	30	24	6	50	1	51
13·5	31	24	7	42	14	56
14	27	22	5	37	15	52
14·5	26	17	9	22	30	52
15	29	11	18	5	42	47
15·5	29	12	17	1	49	50
16	21	8	13	0	40	40
16·5	7	2	5	0	24	24
17	7	0	7	0	27	27
17·5	0	0	0	0	3	3
18	2	0	2	0	1	1
Totals	266	161	105	241	248	489

A=maxillary indices, *i.e.*, the sum of the number of teeth on both maxillae divided by two.

B=aggregates of night catches, comprising window-trap fraction and morning spray-catch fraction.

C=aggregates of window-trap fractions.

D=aggregates of spray-catch fractions.

E=aggregates of collections from casual water.

F=aggregates of collections from permanent water.

G=an artificial frequency distribution constructed by adding the frequencies corresponding to each index in columns E and F.

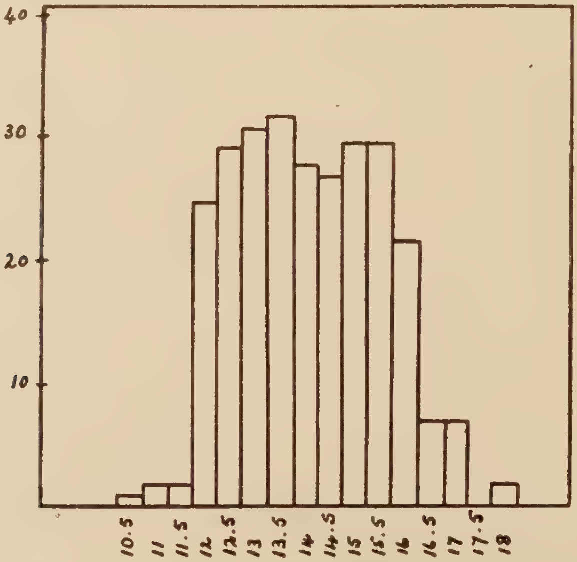

Fig. 2.—Frequency distribution of maxillary indices in aggregate B (Table I).

The frequency histogram above is constructed from columns A and B in Table I. It represents the frequency with which the indices in column A were found in 266 specimens collected from window-traps and spray-catches. It is assumed that any significant departure from normality in this distribution suggests that the population, from which this random sample was drawn, cannot be pure. The normality of the distribution was tested by calculating the first four moments about the mean and the derived constants β_1 and β_2. From these constants were calculated the two further quantities γ_1 and γ_2.

$$\beta_1 = 0{\cdot}02339$$
$$\beta_2 = 2{\cdot}322$$
$$\gamma_1 = 0{\cdot}153$$
$$\gamma_2 = 0{\cdot}678$$

The standard errors of γ_1 (0·1482) and γ_2 (0·3) were also calculated.

It will be seen that γ_1 is slightly larger than its standard error, but that the difference is so small that the lack of symmetry it denotes cannot be considered important. γ_2, however, is more than twice its standard error, indicating that the distribution departs from normality to a degree that must be considered significant. Since β_2 is less than 3 (2·322) the curve of the distribution is platykurtic. Reference to the histogram (fig. 2) shows the departure from normality.

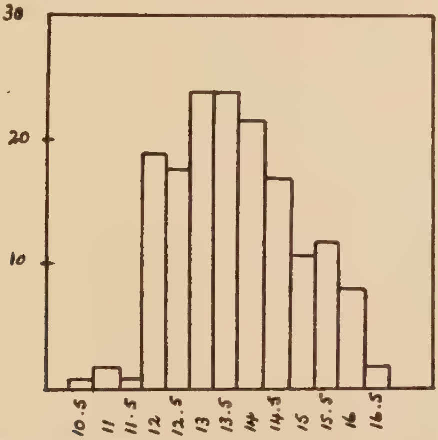

Fig. 3.—Frequency distribution of maxillary indices in aggregate C (Table I).

The histogram in fig. 3 is constructed from the frequency distribution in column C of Table I. It represents the frequency distribution of maxillary indices in the samples obtained from the window-traps.

Standard deviation = 2·502
Mean ... ... = 12·91

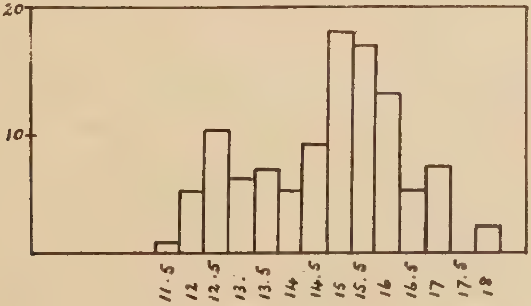

Fig. 4.—Frequency distribution of maxillary indices in aggregate D (Table I).

The histogram above is constructed from the frequency distribution in column D of Table I. It represents the frequency distribution of maxillary indices in the samples obtained from the spray-catches.

Standard deviation= 3·073
Mean ... ... =14·74

To test whether the difference between the means of the two distributions above (figs. 3 and 4) is such that they might have been drawn from the same population, the standard error of the difference between the means has been calculated. The standard error is found to be 0·3479 and, since the difference between the means amounts to 1·83, or more than five times the standard error, the means are significantly different and it is unlikely that they were drawn from the same population.

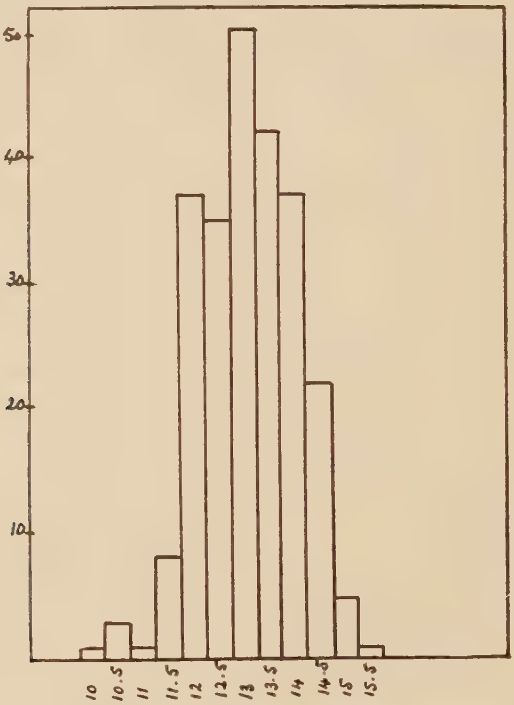

Fig. 5.—Frequency distribution of maxillary indices in aggregate E (Table I).

The histogram, shown in fig. 5, is the frequency distribution of indices in the samples collected over casual rain-water pools, which is given in column E of Table I.

Standard deviation= 1·888
Mean ... ... =13·107

The frequency histogram in fig. 6, below, is that of the samples collected over permanent water in the catchment area of Abuko Water Works. It is the frequency shown in column F of Table I.

Standard deviation= 2·072
Mean ... ... =15·33

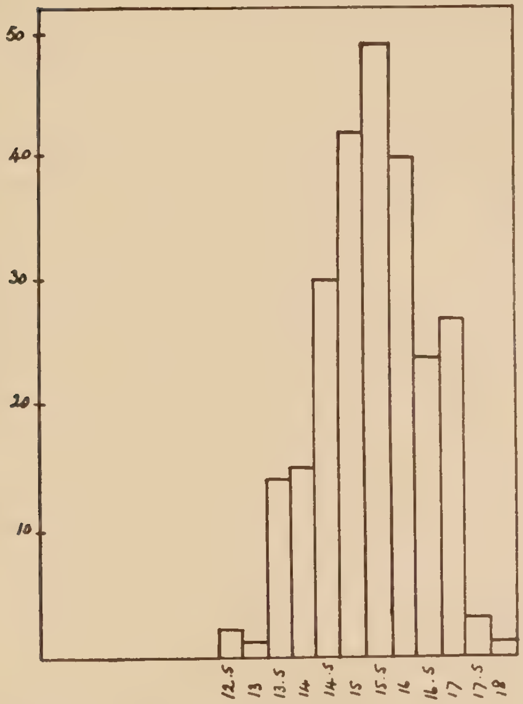

Fig. 6.—Frequency distribution of maxillary indices in aggregate **F** (Table I).

As in the case of frequency distributions C and D (figs. 3 and 4), to test the significance of the difference between the means, the standard error of the difference between the means is calculated and compared with the difference between the means.

Standard error of the difference between the means=0·1792

Difference between means ... ... =2·223

Since the difference between the means (2·223) is nearly 12 times the standard error of the difference between the means (0·1792), it is highly improbable that both samples could have been drawn from the same population, by random sampling.

This frequency diagram has been constructed from the frequency distribution shown in column G of Table I and is derived from the frequency distributions of columns E and F, by adding the frequencies shown in each column opposite each of the indices. This has been done to allow comparison with the histogram in fig. 2. In this case, the two samples collected in tents over the two types of breeding place are combined; in the histogram in fig. 2, the two samples collected from window-traps and spray-catches are also combined. If the samples collected from the window-traps and spray-catches belong to the same two different populations as those collected from casual water and permanent water, we should expect to find that the histograms of the combined frequencies (figs. 2 and 7) resemble one another. The general similarity of these two histograms supports such a hypothesis.

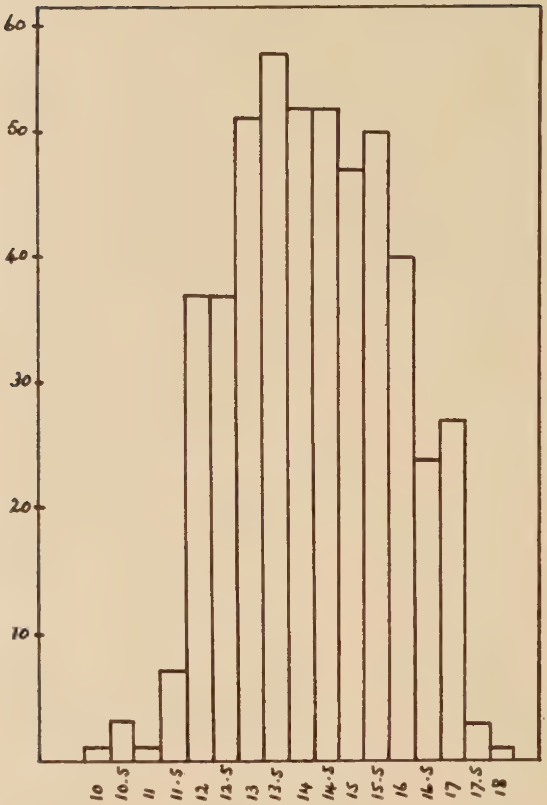

Fig. 7.—Artificial frequency distribution of maxillary indices (Table I, column G).

Once again, as in the distribution shown in fig. 2, the first four moments about the mean are calculated and from them the derived constants β_1 and β_2. From these constants the two further quantities γ_1 and γ_2, are also calculated. In addition, the standard errors of the two latter quantities are calculated. Thus :—

$\beta_1 = 0{\cdot}0007$

$\beta_2 = 2{\cdot}290$

Standard error of $\gamma_1 = 0{\cdot}1108$

$\gamma_1 = 0{\cdot}02645$

Standard error of $\gamma_2 = 0{\cdot}2234$

$\gamma_2 = -0{\cdot}71$

Since γ_2 is more than three times its standard error the distribution departs from normality and, since β_2 is less than 3, the curve of the distribution is platykurtic. The departure from the normal distribution can be seen by reference to the histogram above.

Summary of the analysis.

1. It has been shown that the frequency distribution of maxillary indices in the total control-house catches (*i.e.*, window-trap collections combined with morning spray-catch collections), departs from normality ; the curve being platykurtic and

slightly asymmetrical. With a sample of this magnitude it would be expected that the frequency distribution should not depart significantly from normal if it were drawn from a pure population. Since it does depart significantly from normal we must assume that it is improbable that the specimens collected (*a*) in the window-traps and (*b*) in the spray catches were drawn from the same population.

2. It is accepted that, if the means of random samples are significantly different, it is improbable that the samples could have been drawn from the same population. It has been shown that the mean maxillary indices of (*a*) the window-trap collections and (*b*) the spray-catch collections are significantly different so that we are, therefore, entitled to assume that there is little likelihood that the two samples are drawn from the same population.

3. Similarly, the mean maxillary indices of the samples collected (*a*) over casual water and (*b*) over permanent water are significantly different. The degree of significance, in this case, is so high that we can state with considerable assurance that it is unlikely that the two samples could have been drawn from the same population.

4. When the frequency distributions of the samples collected over the two categories of breeding place are artificially combined by adding the frequencies corresponding to the same indices in both distributions, the resulting frequency curve is not normal, the curve being markedly platykurtic. In this respect it bears a close resemblance to the curve of the combined window-trap and spray-catches described in (1) above and supports the hypothesis that the population being sampled, both by window-trapping and spraying as well as by collecting emerging adults over different types of breeding place, is not a pure population but consists of two races differing in their breeding habits and with maxillary ranges overlapping yet distinct.

Discussion.

Unless it is considered possible that the samples analysed were contaminated by specimens of *A. gambiae* var. *melas*, and the distance of over four miles which separated the catching stations from the nearest saline breeding place, the absence of any four-banded specimens and the physical impossibility that the samples of emergent adults could have been adulterated is strong evidence against this suggestion, it is impossible to avoid the conclusion that the species *A. gambiae*, quite apart from its recognised variety "*melas*", is composed of two distinct races which, however, cannot be differentiated by any constant morphological characteristic. The mean maxillary indices of the two races are, however, quite distinct ; that of the race collected over casual water being 13·107, with a range from 10 to 15·5, and that of the race collected over permanent breeding grounds being 15·33, with a range from 12·5 to 18. It is probable that analysis of still larger samples would show an extension of both ranges.

It is tempting to accept the evidence of the differences between the mean indices of the window-trap and spray-catch collections as showing a difference in behaviour between the two races. It is, however, recognised that there may be an alternative and simpler explanation. The only permanent fresh-water breeding places which exist in the neighbourhood of the catching stations from which the material for analysis was collected, are just over two miles distant whereas, during the rains and for some time after (the period during which the collections were made), casual rain-water pools are abundant and close. It therefore seems reasonable to suppose that a factor of fatigue may operate and that those mosquitos which have travelled far and arrive exhausted with depleted reserves of energy, may remain in houses, where they have fed, in order to recuperate, whilst those that have travelled only a short distance may leave the house soon after they have satisfied their food requirements. Unless this alternative explanation can be disproved, we are not entitled to assume that any differences in behaviour exist. Experience tends to show, in the work of routine mosquito control, that, as a rule, human habitations are further from permanent breeding places than from the numerous collections of casual water which arise everywhere during the rains. How far this theory, which postulates a fatigue factor, can be accepted as explaining conflicting observations on mosquito behaviour, it is not possible to say.

In order to clarify the relationship that appears to exist between the two races and the type of breeding place in which they arise, further research will have to be carried out. The present classification into casual and permanent is convenient but begs the question. Random samples of the micro-flora of breeding places shows that well marked ecological phases give way to one another as the pool ages. No exact work has been carried out but it is possible to list the dominant species in each phase.

TABLE II.

Ecological succession in fresh-water pools.

Phase	Dominant species	Remarks
1	Bacteria	Soil bacteria, aerobic and anaerobic ; saprophytic, colourless.
2	Protozoa (monads)	Microscopic ciliates ; colourless. Larger ciliates.
3	Protozoa	Predacious on both bacteria and ciliates ; colourless.
4	Unicellular green plants ...	Protophyta with chlorophyll. Photosynthesis.
5	Colonial algae	Metaphyta and Metazoa.

In the comparatively few (14) collections of emergent adults which have been made, it is possible to say that the race differentiated by having a mean maxillary index of 13·107 was only found to breed in water of phases 1 to 3, whilst the race differentiated by having a mean maxillary index of 15·33 was only found to breed in water of phases 4 to 5. It was, of course, not possible to exclude rigidly the possibility that either race may have been breeding in small numbers in the other's typical larval habitat, but it is possible to say that each race was dominant in the category given.

Examination of Table II shows that phase 4 introduces photosynthesis and with it, of course, chlorophyll. Thus, in the small series analysed, there is a complete correlation with the presence or absence of chlorophyll. This does not, however, by any means prove that chlorophyll is the factor, whose presence or absence produces adults with large or small mean indices. To establish the reason will call for much further work, involving a study of larval growth and nutrition.

Conclusion.

The work described above, appears to have established beyond reasonable doubt that, amongst fresh-water breeding *A. gambiae* in the Gambia, two races exist which may be differentiated by their mean maxillary indices. There is also good evidence for supposing that each race has its origin in a different type of breeding place ; whilst it is unknown what factor is involved in the variation observed, it has been shown in the small series analysed that the presence or absence of chlorophyll serves to distinguish the two categories of breeding place.

Although, in the observations upon which the statistical analysis is based, it was found that the two races exhibited apparent differences in behaviour, so far as house resting habits are concerned, it is not considered that this point has been proved unless the existence of a fatigue factor, accounting for house resting on the part of adults which have travelled far and exhausted their energy reserves, can be excluded.

The practical value of the work would appear to be confined to the ability to analyse house or other catches into two categories, those from permanent breeding places and those from casual or temporary collections of water. If it can be proved that the races behave differently so far as house resting is concerned it will be a useful addition to our knowledge and must be taken into consideration in assessing the value of residual insecticides and mass spraying. Even if the observed differences in behaviour can be explained on the fatigue factor hypothesis, they must be taken into account in assessing the value of mass spraying.

It is considered that the techniques of statistical analysis could, with advantage, be greatly extended in entomological field work, more particularly in the clarification of problems in connection with insect behaviour. These studies have been confined to variation in maxillary dentition but it may be found that variations in the pharyngeal bar may prove even more fruitful, when examined statistically, although the work will call for more lengthy and careful dissections than can usually be undertaken in the field.

Summary.

Arising out of a routine mosquito survey, carried out in a coastal district of the Gambia in 1946, it was discovered that specimens of typical *A. gambiae* showed a variation in maxillary indices, suggesting the possible differentiation of the species into two races distinct from the known variety "*melas*".

Material from catching stations, considered beyond flight range from possible breeding places of "*melas*", was subjected to statistical analysis as were also collections of adults emerging from different categories of fresh-water breeding places.

The analysis showed that the maxillary indices of the samples obtained from control house catches were distributed in an abnormal manner and were therefore unlikely to have been drawn from the same population. At the same time, the samples collected by window-traps and spray-catches were found to differ significantly in their means, and this supported the view that a mixed population was being sampled. The samples obtained by trapping emerging adults from different categories of breeding places, where any adulteration by "*melas*" was a physical impossibility, showed significantly different mean maxillary indices. It was therefore very unlikely that they could have been drawn, by random sampling, from a pure population. When the samples, obtained by trapping over-breeding places, were divided according to their mean indices, it was found that those falling into the group with a mean index of 13·107 were taken from collections of water which could be classified as casual and those falling into the group with a mean index of 15·33 were collected from permanent pools.

Preliminary research on the micro-flora of fresh-water pools has shown that certain distinct phases, marked by dominant plants, can be distinguished. The distinction between casual water and permanent water is capable of more accurate definition by reference to the dominant species. It has been observed that, within the limits of statistical differentiation, the race having the larger mean maxillary ndex is never found in water where photosynthesis, and hence chlorophyll, is absent whereas the race with the smaller mean maxillary index is never found in water where photosynthesis has been established amongst the micro-flora.

The apparent difference in behaviour between the two races, as shown by the predominance of the 13·107 index group in the window-traps, and the predominance of the 15·33 index group in the spray-catches, cannot it is thought, be accepted as evidence unless the existence of a fatigue factor, accounting for house-resting habits in members of the latter group which have travelled far and exhausted their energy reserves, can be excluded.

It is considered that the results of the work described in this paper may be of value in permitting control house catches to be analysed. From such analysis it should be possible to assess the proportion of adults which had originated in casual water and the proportion coming from more permanent breeding places. If, of course, it should be proved that the apparent differences in adult behaviour are, in reality, linked with differences in mean maxillary indices, it will be necessary to take this into consideration in assessing the value of residual insecticides and mass spraying. It is further argued that, even if the differences in behaviour can be explained on the fatigue factor hypothesis, it will still be necessary to take them into account in attempting to assess the value of mass spraying technique.

It is considered that the method of statistical analysis could be more widely utilised in exploring many problems arising in the field, and that other variations, apart from those of maxillary dentition, might prove even more fruitful as a basis for analysis, although they would call for more time and labour than can usually be spared.

Acknowledgements.

The author wishes to acknowledge his indebtedness to Dr. C. W. F. Mackay, O.B.E., late Director of Medical Services, Gambia, for the opportunity of carrying out this research. He is also indebted to Dr. E. Bury, the present Director of Medical Services, Gambia, for permission to publish this paper.*

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*It had been intended that this paper should not be published until the work had been repeated and a study, using the same technique, had been made of *A. gambiae* var. *melas*. Unfortunately no opportunity for this has occurred and the publication of work on similar lines (Holstein, M. (1949). Le problème d'Anophélès gambiae.—Bull. méd. Afr. occid. franç., **1949**, no. spéc., pp. 155–160), of which the author has, as yet, only seen an abstract, made it seem desirable that this preliminary paper should be published.

FURTHER OBSERVATIONS ON THE BITING-HABITS OF TABANIDAE IN UGANDA.

By A. J. Haddow.

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During the course of field studies on yellow fever in Uganda, a considerable number of 24-hour catches of biting Diptera have been carried out, using human baits. These catches have been designed primarily to investigate the biting habits of mosquitoes, but numerous Tabanids have also been taken. Apart from 24-hour catches, less elaborate methods of collection have also been employed on a large scale, and these also have given some information about the distribution and biting habits of Uganda Tabanidae.

Prior to 1944, all catching was carried out at ground level, but in that year it was concluded that the vector of yellow fever among forest animals in Uganda was probably arboreal, and since then much work has been done in the forest canopy and understorey, almost always with a control at ground level.

During 1944 and 1945, series of catches were carried out simultaneously at ground level and at various heights in forest trees in two localities in Bwamba County, an area which has been described in detail elsewhere (Haddow, 1945a). Forty such catches were made in all (Haddow & others, 1947). Later in 1945, intensive collections of arboreal biting Diptera were made in the same localities over a period of about three months. This work revealed that a Tabanid, *Chrysops centurionis* Austen, is essentially arboreal and crepuscular, biting mainly in the hour following sunset (Haddow & others, 1950).

As catches carried out in the forest canopy with anaesthetised monkeys as bait (Haddow & Dick, 1948) had shown that *C. centurionis* bites monkeys freely, the general conclusion was reached that monkeys are probably the natural hosts, and that *C. centurionis* may be the vector of filariasis which is a very prevalent condition among monkeys in Uganda.

Meanwhile, 40 platforms for use in connection with yellow fever studies were erected in the canopy of the Semliki Forest, which covers much of Bwamba County. During the period 1946–48, a survey of arboreal mosquitoes was carried out from these, six 24–hour catches being made on each with a bait of three Africans, while a control group of three others worked under the tree concerned. These 240 catches greatly increased the sample of *C. centurionis* available for study.

During the same period, seven tree stations were constructed in the Entebbe area, in forest near the shores of Lake Victoria. These stations consisted of a platform in the canopy and one in the understorey. At each of them, a series of five 24-hour catches was made, three Africans working in the canopy, three in the understorey and three at ground level. These 35 catches contributed still further to the sample.

Throughout all this period, small-scale surveys of forested and wooded areas were being carried out in various parts of Uganda in connection with studies on the distribution of yellow-fever vector mosquitoes and on the incidence of the disease among wild monkeys. These surveys always included catches made in the evening in the forest canopy, which revealed that *C. centurionis* occurs over a wide area in Uganda, and that everywhere its habits are crepuscular or nocturnal.

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*This Institute is financed by means of grants by the Secretary of State for the Colonies under the Colonial Development and Welfare Acts and by contributions from the Governments of Uganda, Kenya, Tanganyika Territory, Northern Rhodesia, Nyasaland and Zanzibar.

The number of specimens of this Tabanid taken in 24-hour catches is now large, the total being 453, and it seems worth while to give a further account of its habits based on this large sample, more particularly in view of the current interest in arboreal Tabanids as vectors of loiasis in man (Gordon & others, 1950). In addition, the 24-hour catches in Bwamba have given information about the biting-cycle of another Tabanid, *Haematopota nefanda* Edw. This is a scarce species, of no known medical or veterinary importance, but its biting-behaviour is of a sufficiently unusual nature to warrant attention. Further, a certain amount of knowledge, though of a less detailed nature, has been gained about the habits and distribution of certain other Tabanids in Uganda. The object of the present paper is to discuss the results obtained up to the end of 1949.

Chrysops centurionis Austen.

(*a*) *The Biting-cycle.*

The work here discussed has not revealed any obvious differences in the biting-cycle in so far as level, season or locality is concerned. For present purposes, therefore, the entire sample may be used in discussing the cycle. In this and the following sections, the geometric mean (M_G) worked out to three decimal places has been employed, as this reduces the influence on the final figures of individual unusually large or unusually small catches. In order, however, to give integer numbers and to make for compactness in tabulation, the means have been multiplied by 1,000 in each case. Thus 0·014 becomes 14 and 1·014 becomes 1014. For the sake of brevity, these figures are referred to hereafter simply as 1000 M_G. In many cases, to permit comparisons between different series, they have been reduced to percentages.

The biting-cycle, as shown by the present sample, closely resembles that previously obtained (Haddow & others, 1950). Thus, there is very little biting-activity throughout the day, but just before sunset (17–18 hours) there is a sharp increase, followed in

TABLE I.

The biting-cycle of *Chrysops centurionis.* Entire sample, without reference to locality or level.

Hour	Number taken	1,000 M_G	M_G as per cent.
06–07	3	21	0·7
07–08	1	7	0·2
08–09	2	14	0·5
09–10	3	21	0·7
10–11	3	21	0·7
11–12	3	21	0·7
12–13	3	21	0·7
13–14	4	26	0·8
14–15	2	14	0·5
15–16	7	47	1·5
16–17	8	51	1·7
17–18	63	387	12·5
18–19	145	1,043	33·7
19–20	83	550	17·8
20–21	38	260	8·4
21–22	22	148	4·8
22–23	16	113	3·7
23–24	9	65	2·1
00–01	9	59	1·9
01–02	13	90	2·9
02–03	6	43	1·4
03–04	2	14	0·5
04–05	2	14	0·5
05–06	6	40	1·3

the hour after sunset (18–19 hours) by a very marked peak. The second hour after sunset (19–20 hours) also shows a fairly high level of biting activity, but after this the figures fall rapidly and remain low during the rest of the night (Table I and fig. 1).

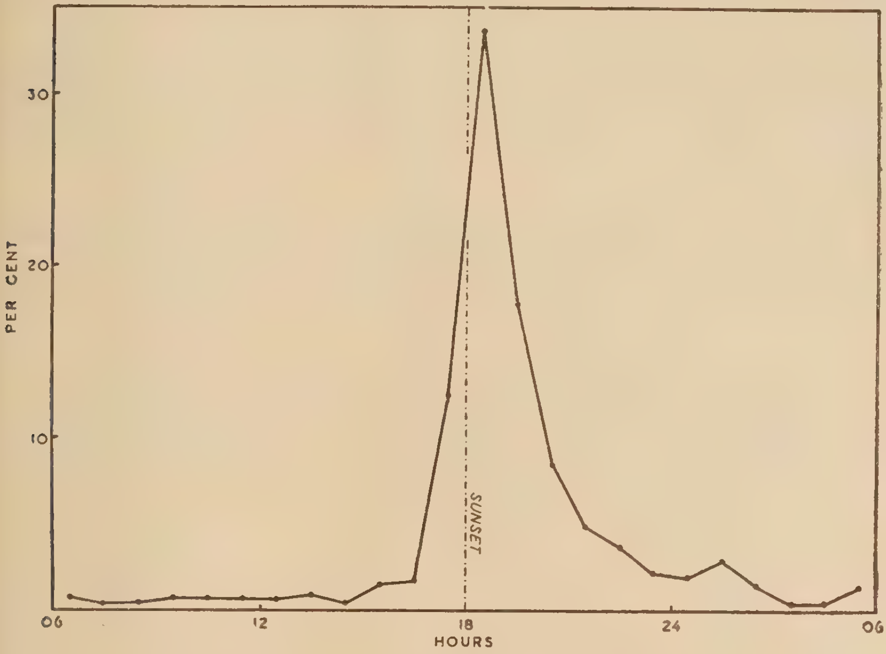

Fig. 1.—The biting-cycle of *Chrysops centurionis.* Geometric means, expressed as percentages of the sum of the means for all periods.

The three important hours may now be considered in more detail. In the period 17–18 hours, 12·5 per cent. of the total catch was obtained, in the period 18–19 hours, 33·7 per cent. was obtained, and in the period 19–20 hours, 17·8 per cent. was obtained. Thus, in the single hour after sunset, more than one-third of the total biting-activity took place, while in the period 17–20 hours, almost two-thirds of the total biting-activity for the whole 24 hours was recorded.

The hour after sunset is, therefore, by far the most favourable for biting. It is also the time at which biting most usually begins, as is shown by the following figures:

Time in hours	Number of days on which biting began at this time	Time in hours	Number of days on which biting began at this time
06–07	3	18–19	37
07–08	1	19–20	6
08–09	1	20–21	5
09–10	2	21–22	0
10–11	3	22–23	3
11–12	2	23–24	1
12–13	3	00–01	2
13–14	2	01–02	1
14–15	0	02–03	2
15–16	4	03–04	0
16–17	2	04–05	0
17–18	17	05–06	1

Thus, while biting may begin at almost any time of the day or night, the important period is again 17–20 hours. *C. centurionis* has been taken by the writer in 98 24-hour catches. In more than one-third of these, biting began in the hour after sunset, and in almost two-thirds it began within the period 17–20 hours. The whole trend of the figures is, in fact, very similar to that of the biting-cycle itself.

It has been found that, in the case of certain mosquitoes, differences in the times at which biting begins on given days may be associated with very different types of biting-cycle (unpublished results). In order to find whether or not this observation applies also in the case of *C. centurionis*, the catches were divided into four groups, namely :

Group A. Twenty-three days on which biting began before 17 hours.
Group B. Seventeen days on which biting began in the period 17–18 hours.
Group C. Thirty-seven days on which biting began in the period 18–19 hours.
Group D. Twenty-one days on which biting began after 19 hours.

TABLE II.

The biting-cycle of *Chrysops centurionis* with reference to the time at which biting-activity began on different days. *Group A*—23 days on which biting began before 17 hours. *Group B*—17 days on which biting began in the period 17–18 hours. *Group C*—37 days on which biting began in the period 18–19 hours. *Group D*—21 days on which biting began after 19 hours.

Hour	Number taken				1,000 M_G				M_G as per cent.			
	A	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*
06–07	3	—	—	—	94	—	—	—	1·4	—	—	—
07–08	1	—	—	—	30	—	—	—	0·4	—	—	—
08–09	2	—	—	—	62	—	—	—	0·9	—	—	—
09–10	3	—	—	—	94	—	—	—	1·4	—	—	—
10–11	3	—	—	—	94	—	—	—	1·4	—	—	—
11–12	3	—	—	—	94	—	—	—	1·4	—	—	—
12–13	3	—	—	—	94	—	—	—	1·4	—	—	—
13–14	4	—	—	—	104	—	—	—	1·5	—	—	—
14–15	2	—	—	—	62	—	—	—	0·9	—	—	—
15–16	7	—	—	—	219	—	—	—	3·2	—	—	—
16–17	8	—	—	—	234	—	—	—	3·4	—	—	—
17–18	33	30	—	—	1,007	1,570	—	—	14·8	49·0	—	—
18–19	52	18	75	—	1,804	710	1,730	—	26·5	22·2	63·9	—
19–20	45	10	19	9	1,492	419	339	284	21·9	13·1	12·5	22·0
20–21	12	6	9	11	364	206	174	353	5·4	6·4	6·4	27·3
21–22	10	2	8	2	269	85	153	68	4·0	2·7	5·7	5·3
22–23	6	2	4	4	183	67	77	140	2·7	2·1	2·8	10·8
23–24	4	1	2	2	128	17	38	68	1·9	0·5	1·4	5·3
00–01	1	3	3	2	30	111	50	68	0·4	3·5	1·8	5·3
01–02	7	—	3	3	219	—	50	104	3·2	—	1·8	8·0
02–03	—	—	2	4	—	—	38	140	—	—	1·4	10·8
03–04	1	—	—	1	30	—	—	34	0·4	—	—	2·6
04–05	—	1	1	—	—	17	19	—	—	0·5	0·7	—
05–06	3	—	2	1	94	—	38	34	1·4	—	1·4	2·6

The results (Table II and fig. 2) show that on days when biting began before 17 hours the peak is rather ill defined, being spread over the period 17–20 hours, with a maximum in the hour after sunset. On days on which biting began in the period 17–18 hours, there is a marked peak of activity in the hour before sunset, after which the figures are much reduced. In the hour after sunset, however, they are still

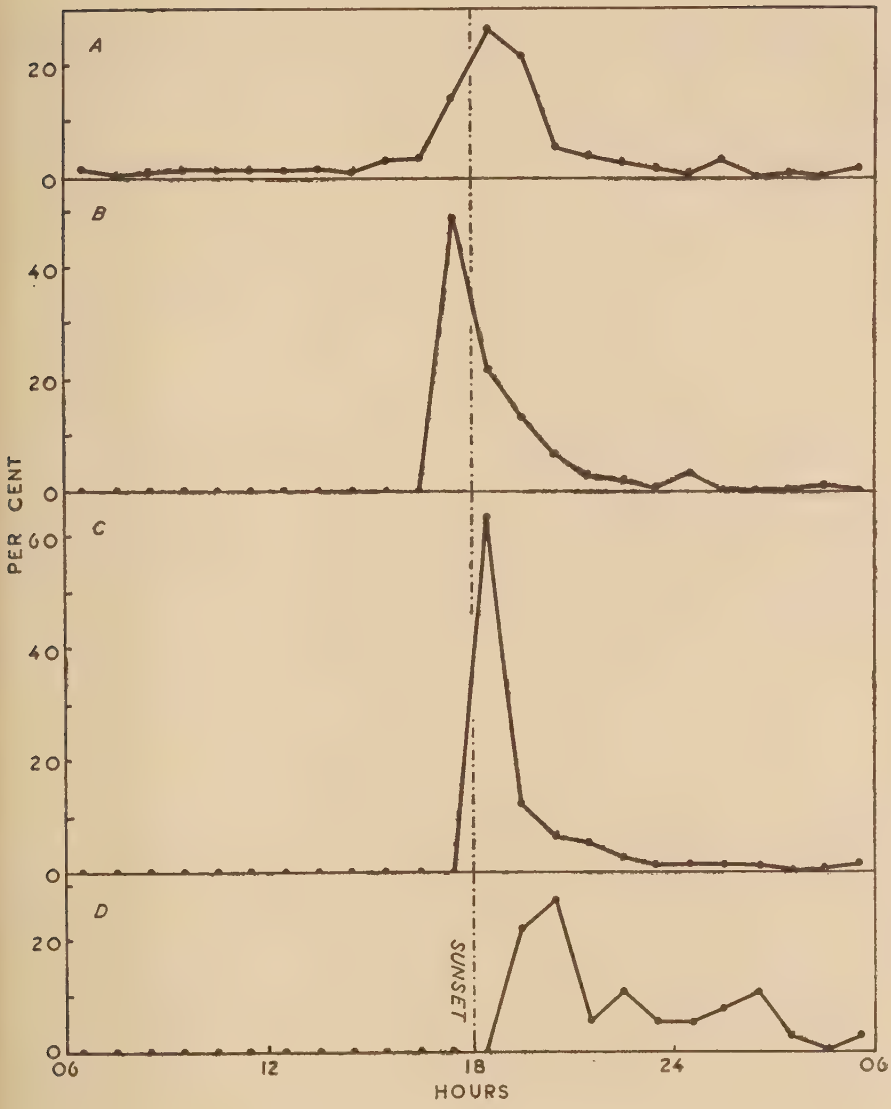

Fig. 2.—The biting-cycle of *Chrysops centurionis*. *A*—days on which biting began before 17 hours. *B*—days on which biting began between 17 and 18 hours. *C*—days on which biting began between 18 and 19 hours. *D*—days on which biting began after 19 hours. Geometric means, expressed as percentages of the sum of the means for all periods.

relatively high and, in this group, the main phase of biting-activity may be considered as spread over the two-hour period 17–19 hours. On days on which biting began in the period 18–19 hours, the peak is exceedingly well defined, and the main phase of activity may be considered as confined to this single hour. Finally, on days when biting activity began after 19 hours, the peak is ill defined and covers the period 19–21 hours; in general form it is not unlike that obtained on days when biting began before 17 hours, but instead of covering a three-hour period it is cut short after two hours, presumably because the favourable period following sunset has ended. It will be noted, however, that in this last group the smallness of the peak is compensated for by a relatively higher level of activity during the rest of the night than occurs in the first three groups.

In an attempt to analyse the four peaks more closely, the percentages of the total catch which they make up in the different groups may be compared as follows :

Group	Period covered by biting peak	Percentage of total catch taken during the peak
A	17–20 hours	63·2
B	17–19 ,,	71·2
C	18–19 ,,	63·9
D	19–21 ,,	49·3

It will be noted that the first three figures are very closely grouped ; this seems to indicate that some particular section of the population forms the main contribution to the evening peak of activity. The figure for the fourth group is much lower, this again suggesting that the peak in this case is an abortive one, cut short by unfavourable conditions.

In order to find whether the most favourable days for catching are those on which biting-activity began early or late, the geometric mean catch per day has been worked out for the four groups, the results being as follows :

Group	Mean catch per day (1,000 M_G)
A	8,120
B	3,169
C	2,651
D	1,601

Thus the yield tends to be largest on days when biting begins early and becomes progressively smaller the later such activity begins.

Another aspect of the problem concerns the duration of biting-activity on different days. Thus, in some cases, all biting-activity may occur within a single hour, while in others it may extend almost throughout the whole twenty-four. If the figures be analysed without reference to clock time, but simply with regard to the duration of activity, some interesting points emerge. Before these are discussed, however, it must be mentioned that a period of activity, as here defined, does not necessarily imply that such activity was continuous. Thus, a day on which biting females were taken during three successive hours would be given the same rating as a day on which they were taken in the first and third but not in the second hour, both being rated as three hours. The results are as follows :

Duration of biting-activity	Number of days on which activity of this type was recorded
1– 3 hours	56
4– 6 ,,	13
7– 9 ,,	9
10–12 ,,	8
13–15 ,,	6
16–18 ,,	4
19–21 ,,	1
22–24 ,,	1

The general tendency is, therefore, towards a very short phase of biting-activity on any given day. Actually, the commonest type of cycle was that in which all biting was confined to a single hour, this applying to 37 days, or more than one-third of the total experience.

A final point for consideration concerns the intensity of biting-activity in relation to the duration of that activity. In other words, does the duration of activity on a given day tend to bear some relationship to the size of the catch? If the geometric means for entire days are considered, the following results are obtained :

Duration of biting-activity	Mean catch per 24 hours (1,000 M_G)
1– 3 hours	1,780
4– 6 ,,	3,295
7– 9 ,,	7,072
10–12 ,,	12,300
13–24 ,,	8,909

Thus, the catch per day tends to increase steadily until the periods of biting-activity have become long (10–12 hours), after which it falls off again. Obviously, however, until the 10–12 hour type of activity is reached, the increase in yield is gradual, and might be accounted for simply by the increase in the duration of activity. It is only by considering the figures on an actual hourly basis that an answer can be given as to whether, with reduction in the periods of activity, biting becomes more intense, to compensate for that reduction. The results are as follows :

Duration of biting-activity	Mean catch per hour (1,000 M_G)
1– 3 hours	977
4– 6 ,,	352
7– 9 ,,	294
10–12 ,,	253
13–24 ,,	153

Thus the intensity of biting-activity, as expressed by the geometric-mean catch per hour, decreases steadily as the whole cycle of activity becomes progressively longer. The figures in the preceding text-table, however, show that this decrease is more than counterbalanced by the increase of yield with time, until the total duration of activity has exceeded 10–12 hours.

(*b*) *Vertical Distribution.*

In the previous communication on this subject (Haddow & others, 1950), the vertical distribution of *C. centurionis* in the Semliki Forest, Bwamba, was discussed in detail. It was shown that during the rains this species bites predominantly in the forest canopy but that, during the dry season, the understorey is the preferred level. During the 40 24-hour catches concerned (carried out during 1944 and early 1945) only one specimen was taken at ground level, as opposed to 140 in the trees.

Later in 1945, during a short period of intensive catching in the evenings on high tree-platforms, with controls at ground level, a further 150 were taken in the canopy, as opposed to 17 at ground level. This is the only occasion on which a substantial number have been obtained at ground level, and it is thought that these catches, made during April, probably coincided with a period of peak incidence of *C. centurionis*. Slightly later in the same year, a further series of platform catches, made without ground level controls, yielded a further large sample from the canopy and understorey.

During the period 1946–48, the 240 catches in the canopy with controls at ground level yielded 218 specimens from the canopy, none being taken at ground level. Similarly, the 35 24-hour catches carried out in the Entebbe area yielded 94 specimens from the canopy and understorey, none being obtained by the ground level controls.

It may further be mentioned that, in the various other Uganda localities in which *C. centurionis* has so far been found (see below), all the specimens have been taken in trees, not one having been obtained at ground level.

In all, between 1944 and the time of writing (1950), the writer and colleagues have collected in the forest canopy and understorey nearly 3,000 *C. centurionis*, the total number obtained at ground level during the longer period 1942–50 being under 50.

The vertical distribution of *C. centurionis* in Bwamba need not be discussed further. It remains to describe the distribution in the lighter rain-forest around Entebbe. The small lake-shore forests of this area have, on the whole, a rather sparse and irregular canopy and a poorly defined understorey. They are thus more open to the penetration of sun and wind than are the heavy forests of the Western Province.

In spite of these differences between Bwamba and Entebbe, the vertical distribution of *C. centurionis* appears to be very similar in both areas, the Entebbe figures being as follows :

Level	Number taken	Mean catch (1,000 M_G)	M_G as per cent.
Canopy	71	1,118	77·4
Understorey	23	324	22·6
Ground level	0	0	0

As, in the Entebbe area, there is usually a lake breeze during the day which is reversed in direction and very often reduced in force by night, it was thought that (for this and other reasons) the vertical distribution of *C. centurionis* might not be the same during the day as during the night. The figures, however, show that there is no difference in this respect :

Level	Number taken		Mean catch (1,000 M_G)		M_G as per cent.	
	Day	Night	Day	Night	Day	Night
Canopy	12	59	199	981	76·5	74·9
Understorey	3	20	61	328	23·5	25·1
Ground level	0	0	0	0	0·0	0·0

Another point of importance concerns the number of days on which *C. centurionis* was taken at the various levels. In the 240 catches made during 1946–48 in Bwamba (where none was taken at ground level), this species occurred in the canopy on 49 days. At Entebbe, where 35 catches were made, it occurred in the canopy on 19 days and in the understorey on 9 days. These catches were carried out at all times of year, and in this connection it must be emphasised that, while *C. centurionis* does occur throughout the year, it is only really prevalent during the main rains, roughly from April till July, with a peak of incidence early in this period.

With regard to its distribution within the areas here discussed, it may be noted that it was taken at 18 of the 40 Bwamba tree-stations, and at six of the seven Entebbe tree-stations. It is also worth noting, however, that while at Entebbe it occurred in the canopy in six stations, it was taken in the understorey in three only.

(*c*) *Discussion.*

Recent work on loiasis (Gordon & others, 1950) raises many issues concerning *C. centurionis*. These workers have shown that the distribution of the disease corresponds very closely to that of the equatorial rainforest, as does also the known distribution of the insect vectors, *Chrysops silacea* Austen and *C. dimidiata* Wulp. The main distribution of the disease reaches its eastern limit in the eastern Belgian Congo, just where *C. centurionis* replaces these species which are closely allied to it. There is, indeed, an infected area in the Sudan, but here the vectors appear to be quite different (Woodman, 1949). On the other hand, D. J. Lewis (personal communication) has pointed out that *C. silacea* does occur in the extreme south of the Sudan at Sources Yubu (Tambura) just north of the Congo border.

C. silacea and *C. dimidiata* are, like *C. centurionis*, essentially arboreal, as has been shown by Gordon and his co-workers. A very important difference, however, is that they are apparently entirely diurnal. The periodicity of microfilariae of *Loa loa* (Cobbold) in the peripheral circulation also being essentially diurnal in most areas, these two species are obviously much better fitted to act as vectors than the crepuscular or nocturnal *C. centurionis*.

A further point of interest arises from the view advanced by Gordon & others that monkeys may be involved in the epidemiology of loiasis and that when man is not available they may act as the chief hosts of *C. silacea* and *C. dimidiata*. Monkey filariasis is prevalent in Uganda and it has already been suggested that *C. centurionis* may be the vector (Haddow & others, 1950). It has also been shown that this species will bite anaesthetised monkeys in the forest canopy (Haddow & Dick, 1948). In this connection it must be considered as a possibility that loiasis in monkeys (should the filariae obtained from monkeys in West Africa be confirmed as *L. loa*) may extend further to the east than the human disease, as specimens of *C. centurionis*, biting in the late afternoon, might act as vectors. Even so, it seems unlikely that *C. centurionis* could be involved in the epidemiology of the human disease, for although Gordon and others have shown that, where forest undergrowth has been cleared, *C. silacea* and *C. dimidiata* will descend from the canopy to bite man at ground level in large numbers, long experience of *C. centurionis* has shown that it does not behave in this way, biting at ground level being quite exceptional, even where survey lines and camp sites are being cleared in forest ; in any case, its crepuscular habit implies that it is active mainly at a time when Africans are rarely to be found in the forest. Moreover, even where plantations are actually contiguous with forest, *C. centurionis* does not seem to enter them, and while *C. silacea* and *C. dimidiata* bite readily in houses, *C. centurionis* does not, the writer having only a single record of a female biting indoors.

Here, therefore, as in the case of yellow fever (Mahaffy, 1949), the epidemiology of a human disease appears to be closely bound up with the habits and distribution of certain blood-sucking insects, a difference between the times and levels of biting-activity possibly proving a crucial factor in the transmission of infection. It must, however, be emphasised in this connection that our present knowledge of the biting habits, distribution and host-preferences of the African Tabanids is exceedingly scanty, and that further work is needed.

Males of *C. centurionis*, like those of *C. silacea* and *C. dimidiata*, have not been found in nature, and larvae have not as yet been sought. In view of the work of Gordon & others, it seems likely that, as in the case of *C. silacea* and *C. dimidiata*, the breeding places may prove to be small forest streams.

Haematopota nefanda Edw.

So far the writer has encountered this scarce species only in the Semliki Forest, Bwamba County. To date, 40 specimens have been taken in 24-hour catches, 38 at ground level and two in the canopy.

H. nefanda has obviously a very short season and so far, with the exception of one specimen, it has been taken only during the main rains. Thus 13 were obtained at the end of May 1947, all at one station. No further specimens were taken till the beginning of June 1948. During this month, 26 were collected, at nine different stations. The single specimen taken in a different season was obtained in September 1946, during the short rains.

Though the sample is small, the biting behaviour seems to be well defined, and as the cycle is of an unusual nature, it is discussed briefly below.

Both specimens taken in the canopy were obtained after nightfall, one between 19 and 20 hours and the other between midnight and 01 hours. These specimens are included with those taken at ground level, to give a total sample of 40. In considering the biting-cycle of this species, it is convenient to take midnight instead of dawn as the starting point, as activity is centred around sunrise and sunset. The results are shown in Table III and fig. 3.

TABLE III.

The biting-cycle of *Haematopota nefanda.*

Hour	Number taken	1,000 M_G	M_G as per cent.
00–01	1	35	2·8
01–02	—	—	—
02–03	—	—	—
03–04	—	—	—
04–05	5	122	9·7
05–06	12	339	27·0
06–07	4	132	10·5
07–08	5	172	13·7
08–09	4	148	11·8
09–10	—	—	—
10–11	—	—	—
11–12	—	—	—
12–13	—	—	—
13–14	—	—	—
14–15	—	—	—
15–16	1	35	2·8
16–17	4	132	10·5
17–18	2	72	5·7
18–19	1	35	2·8
19–20	1	35	2·8
20–21	—	—	—
21–22	—	—	—
22–23	—	—	—
23–24	—	—	—

Thus, *H. nefanda* begins biting, very abruptly, two hours before sunrise, reaches a peak in the hour before sunrise, and continues to bite actively for a further three hours, after which biting-activity ceases as abruptly as it began. There is no further

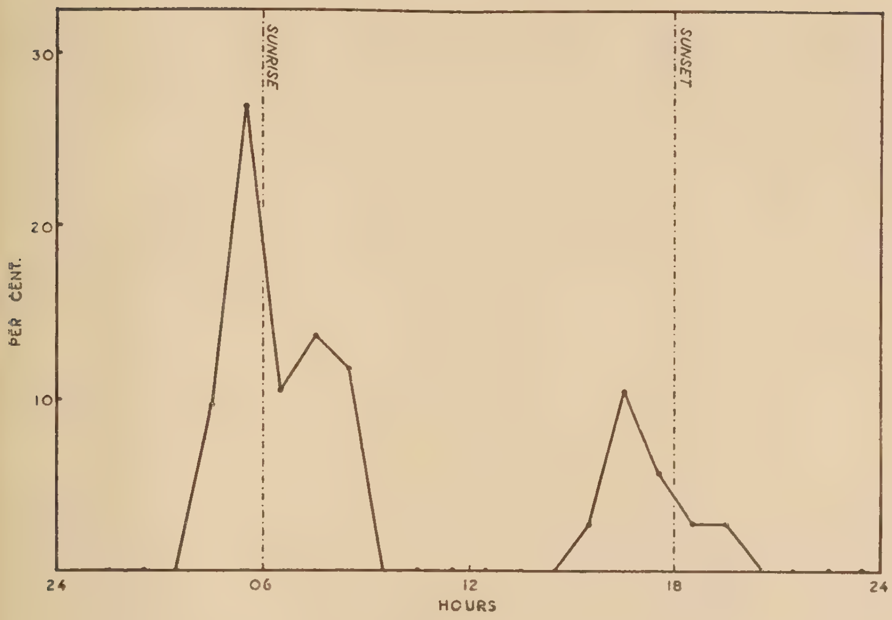

Fig. 3.—The biting-cycle of *Haematopota nefanda*. Geometric means, expressed as percentages of the sum of the means for all periods.

biting till late in the afternoon, when a moderate peak is reached between 16 and 17 hours. In the succeeding hour, that before sunset, activity is already diminished, and very little biting takes place after sunset.

This biting-cycle is unique in the writer's experience, the two peaks falling at periods which, climatically, bear no resemblance to each other. Thus, in the period of the first peak, 05–06 hours, temperature and saturation deficiency have reached their minima, relative humidity is at its maximum, and light intensity is exceedingly low. In the period of the second peak, 16–17 hours, temperature and saturation deficiency have just passed their maxima, relative humidity is exceedingly low, and light intensity is considerable.

In an earlier paper (Haddow, 1945b), the writer introduced the term "eo-crepuscular" to describe the behaviour of mosquitoes which exhibited two peaks of activity in the 24 hours, these peaks being associated with sunrise and sunset. In all such cases, however, the behaviour has been quite different from that of *H. nefanda*. Thus in the case of a diurnal species the peaks would be after sunrise and before sunset, while in a nocturnal species they would be after sunset and before sunrise.

The biting-activity of *H. nefanda*, therefore, cannot be explained on microclimatic grounds alone. It seems necessary to make a further postulate, namely, that there are two sections in the population with entirely different microclimatic responses. These might be, for example, different age-groups, or ovipositing and non-ovipositing females, or first and second blood-meal groups. Only further work, combined with dissections, can elucidate this point.

Notes on other Species.

Information concerning the biting habits of the other species so far encountered in Uganda by the writer is not adequate for detailed discussion and can only form short notes, together with locality records. Some notes have already been given in the case of nine Bwamba species (Haddow & others, 1950). In these cases, for the sake of brevity, only new behaviour records are given here.

A most interesting point is that several of the species concerned have shown evidence of activity in the crepuscular period or by night, as follows :

Genus	Species observed	Species exhibiting crepuscular or nocturnal activity
Tabanus	13	5
Haematopota	9	4
Hippocentrum	1	0
Tabanocella	1	1
Chrysops	5	2
Totals	29	12

Dr. J. Bequaert of the Museum of Comparative Zoology, Harvard, has pointed out (personal communication) that similar habits occur also in the case of American Tabanids. He notes that " Nocturnal habits are probably far more common in Tabanids than is realized, particularly in the tropics, no doubt correlated with the nocturnal mammals. In America several species of *Chlorotabanus*, with a characteristic grass-green colour, are decidedly crepuscular (*C. mexicanus*, *C. inanis*, etc.). In Colombia I observed *Dichelacera vespertina* at dusk and after dark in dense forest glades, biting horses. It would come on noiselessly and settle on the belly of the horse between the hind legs where it was difficult to catch, except by hand. The very peculiar *Bolbodimyia bicolor* had the same habit in that locality."

Certain of the records given below are contributed by a colleague, Dr. W. H. R. Lumsden ; these are acknowledged as (L.). Where possible, the eye-colours are described.

Tabanus ditaeniatus Macq. Bwamba County (Toro District.).

Nothing to add to previous records.

T. fasciatus niloticus Aust. Jubiya Forest (Masaka District) ; Sese Islands (Masaka District) ; Busi Island (Mengo District).

This is a very common species around the shores of Lake Victoria and its islands. It settles in numbers in launches and canoes but has not so far been observed biting man. It seems to spend much of its time out over the shallows in bright sunshine, being most prevalent in reedy areas. On one occasion, a hippopotamus, which had been shot about 12 hours previously, was brought ashore at Sese. Large numbers of *T. fasciatus niloticus* at once alighted on it and began to bite. Some managed to engorge on black, viscous blood. This species having shown such eagerness in attacking a cold carcase, it was concluded that hippopotamus may be the main host, though probably elephant and buffalo drinking or bathing are also attacked.

In life the whole body is suffused with a fresh grass green, the colour being due to that of the insect's blood. The eyes are brilliant emerald green.

T. marmorosus var. *congoicola* Beq. Bwamba County.

One female, not biting, has been taken in a house by day. The eyes were very dark Hooker's green.

T. neavei Aust. Entebbe area (Mengo District).

One female, not biting, has been taken in a house by day. The eyes were purplish-black, with one green band.

T. par Wlk. Bwamba County ; Entebbe area.

Additional records for this common species are as follows. It has been taken biting in the forest canopy by day (L.) and at ground level in forest by night (L.). One female, not biting, has been taken in a house by day. The eyes are brilliant emerald green.

T. ruwenzorii Ric. Ruwenzori Mountains (Toro District).

This species is very common in the grasslands just below the mountain forest of the Nyamugasani Valley, at 6,000 to 7,000 ft. It has not been observed to bite man. On one occasion it was observed settling in numbers on greenish-khaki nylon tents and repeatedly probing the material. These small tents were presumably taken to be grazing animals.

The eyes are dark brown.

T. secedens Wlk. Bwamba County.

One female, not biting, has been taken in a car in ironwood (*Cynometra*) forest (L.). The eyes were very dark green, with purplish reflections.

T. socialis Wlk. Bwamba County.

This rather scarce species has been taken in the forest canopy both by day and by night (L.). One female, not biting, has been taken in a house by day (L.).

T. taeniola P. de B. Bwamba County ; Lake Nakivali (Ankole District).

Additional records are as follows. One male has been taken in the forest canopy by night (L.), and one female has been observed biting a newly shot topi (*Damaliscus korrigum ugandae* Blaine) in savannah country in the afternoon.

T. thoracinus P. de B. Lake Nakivali ; Entebbe area ; Jubiya Forest ; Mubende area (Mubende District).

This species has been taken biting at ground level in forest by day. Females, not biting, have been taken in a car, and in a house and a tent by day. Males have been taken in the forest canopy by night.

The eyes of the female are a brilliant emerald green. Those of the male are greenish-yellow above and deep emerald green below.

Tabanus sp. aff. *variatus* Wlk. Soroti area (Teso District).

One female has been taken biting at ground level in bush, by day. This specimen has been deposited in the British Museum.

T. williamsi Aust. Bwamba County.

Nothing to add to previous records.

T. xanthomelas Aust. Bwamba County.

Nothing to add to previous records.

Haematopota brucei Aust. Bwamba County.

Nothing to add to previous records.

(All the species of *Haematopota* so far examined have purplish-black eyes with bands of bronze-green. This applies to females only.)

H. edax Aust. Mubende area.

Females have been taken biting at ground level by day in dry forest.

H. furva Aust. Bwamba County ; Ruwenzori Mountains ; Rubanda area (Kigezi District).

H. furva is exceedingly common in the Ruwenzori grasslands below the mountain forest, and it also occurs within the lower edges of the forest itself, its vertical range being from about 5,000 to about 7,000 feet. It bites man viciously by day, usually in strong sunshine. It has also been taken biting by day on the edge of a papyrus swamp in Kigezi District, at a height of over 6,000 feet. Like *T. ruwenzorii*, it attacks tents in numbers. Though one female has been taken biting at ground level by day in the lowland forest of Bwamba County, this record is an exceptional one and, generally speaking, *H. furva* seems to be a Tabanid of the high grasslands.

H. guineensis Bigot. Bwamba County.

This species has been taken only in a single area of swampy forest (Mongiro), where it is evidently very scarce. It has been taken biting at ground level by day and also in the canopy by day (L.) and by night (L.).

H. hirta Ric. Rubanda area.

Females have been taken biting by day on the edge of a papyrus swamp, at a height of over 6,000 feet, in company with *H. furva.*

H. nefanda Edw. Bwamba County.

Apart from the above records of biting habits, it may be noted that two males believed to belong to this species have been obtained, one in forest undergrowth by day and the other in a house by day.

The eyes of these males differed completely from those of the female. They were exceedingly large and of a pale sky blue throughout, with no trace of banding.

H. patellicorne (End.). Bwamba County ; Kalinzu Forest (Ankole District).

In the previous paper (Haddow & others, 1950) this species was referred to *H. vittata* (Lw.). Additional observations on the habits of this species are that it frequently follows and enters cars on forest roads, and bites freely around stationary cars in this environment.

Haematopota ? sp.n. Ruwenzori Mountains.

This species was obtained with *H. furva* and *T. ruwenzorii* in the high grasslands and lower mountain forest of the Nyamugasani valley, biting by day.

The material has been deposited in the British Museum.

Hippocentrum strigipenne (Karsch). Bwamba County.

An additional observation on this species is that it will bite in and around cars within the main forest. It presumably follows them along the forest road from its main habitat in the swampy areas just outside the forest edge.

The eyes are similar to those of *Haematopota* females.

Tabanocella perpulchra (Aust.). Bujuko Forest (Mengo District).

Several were taken alighting on a stationary car at dusk. They made no attempt to bite. The car was in a banana plantation on the forest edge.

Chrysops centurionis Aust. Bwamba County ; Kibale Forest (Toro District) ; Bugoma Forest (Bunyoro District) ; Kalinzu Forest ; Entebbe area ; Kampala area (Mengo District) ; Mukono area (Mengo District).

This species has also been taken in West Nile District by Lumsden and Buxton (1951).

The eyes of *C. centurionis* (and also of the other species of *Chrysops* mentioned below) are purplish-black with bronze-green bands, rather as in *Haematopota* spp., though when looked at from a slight distance they appear golden-brown.

C. distinctipennis Aust. Lake Nakivali.

One female was taken biting man at ground level in savannah in bright sunshine, and another biting a newly shot reedbuck (*Redunca redunca ugandae* (Blaine)) in the same habitat.

C. funebris Aust. Bwamba County ; Entebbe area.

Females have been taken biting at ground level in forest by day, in the canopy both by day and by night, and in open country by day. They have also been taken, though not biting, in houses by day.

C. griseicollis Beq. Bwamba County.

Previous records of this rare species (Haddow & others, 1950) referred to three specimens, all taken in the forest understorey and canopy in the afternoon, during the dry season. A further record has been obtained of one female, also taken biting in the canopy in the afternoon, and again in the dry season (L.). All four specimens came from the same forest area (Mongiro).

C. longicornis Macq. Bwamba County.

Nothing to add to previous records.

In the previous paper on this subject (Haddow & others, 1950) *C. funebris* was referred to as *C. longicornis* v. *funebris*. The concensus of opinion, however, seems to be that *funebris* and *longicornis* are distinct species.

Acknowledgements.

The writer wishes to thank Mr. H. Oldroyd of the British Museum (Natural History) who has confirmed the identification of almost all the material here discussed, and Dr. W. H. R. Lumsden, who has kindly contributed several records of Uganda species.

Summary.

The biting habits of *Chrysops centurionis* Austen and its distribution in Uganda are discussed. This species is everywhere arboreal and mainly crepuscular. The absence of loiasis in man in Uganda is, it is suggested, due to differences between the biting habits of this species and those of the known vectors, *C. silacea* Austen and *C. dimidiata* Wulp, which are closely allied to *C. centurionis*.

The peculiar biting-cycle of another Tabanid, *Haematopota nefanda* Edw., is described briefly.

Short notes on the biting habits and distribution of some other Uganda Tabanids are given.

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FIELD TESTS OF DRY BAITING AGAINST THE DESERT LOCUST, *SCHISTOCERCA GREGARIA* (FORSK.).

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Poisoned bait seems to have been first used against locusts by Coquillet in 1885, although experiments had been done as early as 1878 (Shotwell, 1942). Instead of spraying arsenical solutions on the vegetation, which damaged it and also made it useless until rain had washed away the arsenic, Coquillet made bait of chopped vegetation soaked in the solution (Douence, 1929) ; later he used six parts of bran and one of arsenic, with one part of sugar in solution to make the arsenic stick (Riley, 1891). Baiting became the most economical and widely used method of destroying locusts and is the subject of voluminous literature reviewed by Uvarov (1928), Douence (1929), Parker (1934), du Plessis & Nolte (1941) and Shotwell (1942). It is only during the past five years that another method of control—spraying or dusting of the insects and the vegetation with the new organic insecticides—has begun to compete with baiting (Shotwell, 1949 ; Wakeland & Parker, 1949). The method to choose to-day depends on the circumstances, but there is certainly still considerable scope for baiting. When, therefore, it became apparent in 1949 that a new plague of the Desert Locust, *Schistocerca gregaria* (Forsk.), was beginning, arrangements were made for trying new and improved methods of control, including improvements in the composition and methods of application of poisoned bait.

The experiments were begun in British Somaliland in March–April 1950, at the invitation of Mr. P. R. Stephenson, Director of the Desert Locust Survey. He was particularly interested in control methods which require neither the standard carrier,

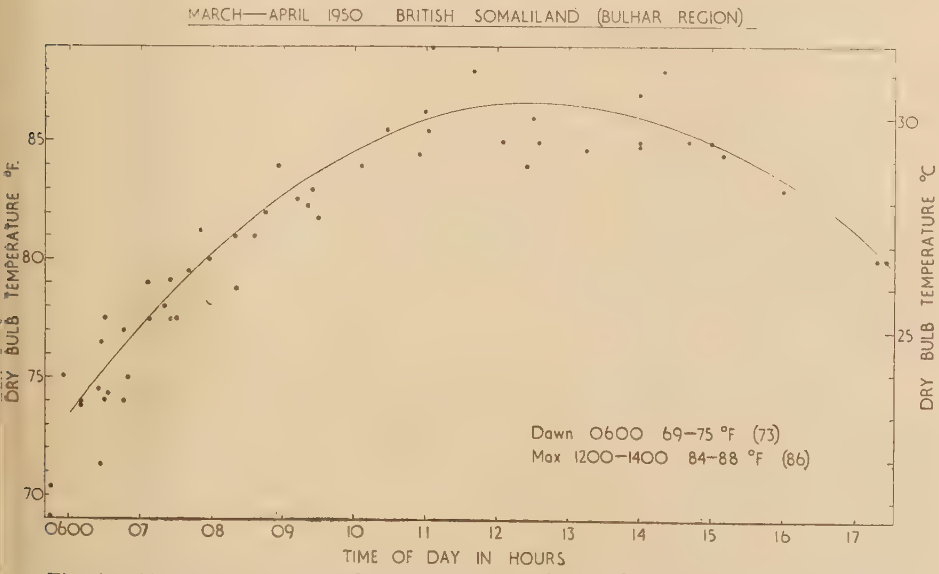


Fig. 1.—Air temperature records are shown as points and the line has been drawn freehand. Bulhar is on the shore of the Gulf of Aden at about 10° 23′N., 44° 25′E. ; the experiments were done within 25 miles of it between the east and south-west.

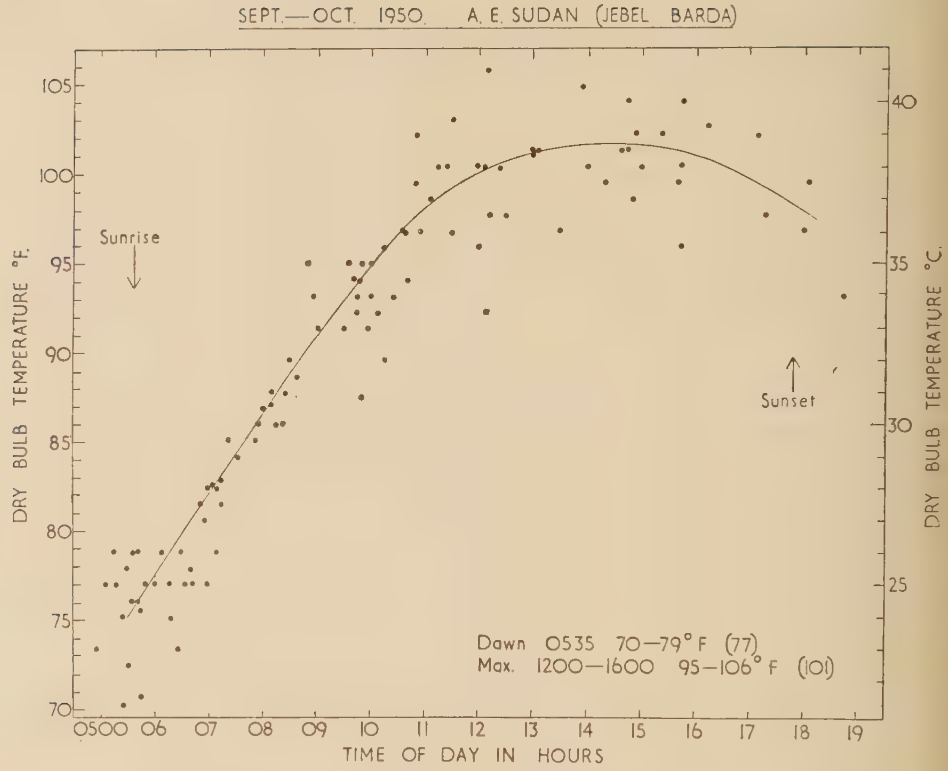


Fig. 2.—See legend to fig. 1 ; to accommodate the greater fluctuation of temperature, the scale of this figure is lower. Jebel Barda is in the desert some 22 miles N.W. of Shendi at about 16° 57′N., 33° 13′E. ; the experiments were done within 10 miles of it to the south and west.

wheat bran, because of the difficulty of obtaining sufficient quantities of it, nor water, a commodity which is frequently troublesome, and sometimes impossible to get in the places where control of the Desert Locust should be carried out. The experiments were continued in the Anglo-Egyptian Sudan in September–October, 1950, at the invitation of Mr. R. C. Maxwell-Darling (Chief of the Research Division) and Mr. Vernon Joyce (Government Entomologist), the emphasis being shifted to high-speed mechanised methods. The present paper deals only with bait tests and not with the tests of spraying and dusting which were done during the same periods.

Weather.

In both Somaliland and the Sudan, the main experiments were done on locust hoppers hatched from eggs laid during the rainy season that had just passed. In Somaliland there was very little rain during the work and in the Sudan only two showers, though those were heavy and violent ones.

Temperatures and humidities were estimated with a mechanically ventilated wet-and-dry bulb hygrometer (Assmann) held at shoulder level. The daily course of temperature change is indicated in figs. 1 and 2. In Somaliland, dawn temperatures were around 24°C., rising to a maximum of about 30°C. by noon and maintaining that level until about 3 p.m. In the Sudan, dawn temperatures were slightly higher at around 25°C., while the daily maximum was much higher at about 38°C., occasionally rising to 41°C. The sand surface in sunshine was often obviously much hotter, sometimes too hot to touch with comfort.

Air humidity was usually high in the early morning in Somaliland, and heavy dew was seen on metal on several mornings. Fig. 3 shows that there was a slight tendency for the water vapour pressure to *rise* as the air became hotter, but on the whole it remained remarkably constant at about 20 mm. Hg. and the relative humidity seldom fell below 60 per cent.

In the Sudan, on the other hand, the vapour pressure was seldom as high as 20 mm. and it went below 6 mm., with a tendency to *fall* as the temperature rose (fig. 4). Consequently a relative humidity as high as 60 per cent. was experienced only in the early morning, and then not often, and at midday it was commonly as low as 15–20 per cent.

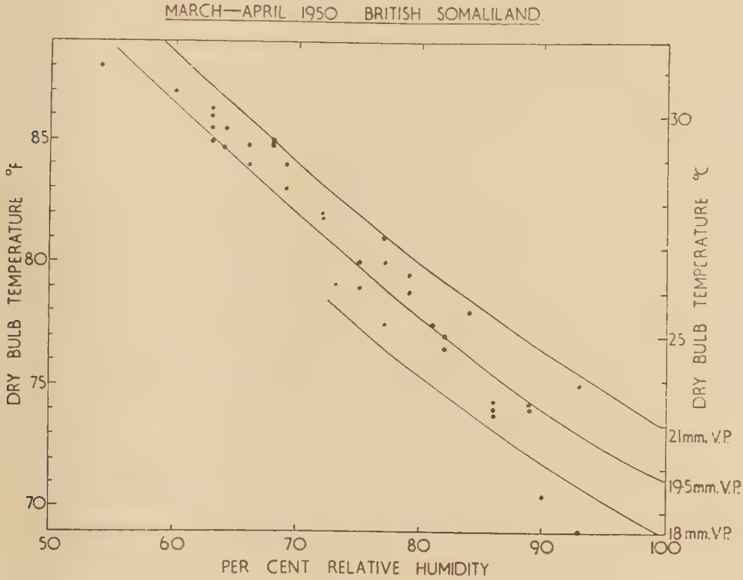


Fig. 3.—Relative humidity records are shown as points. The lines represent approximately constant amounts of water vapour in the atmosphere. The relative humidity was high at the low temperatures of dawn and rarely fell below 60 per cent. R.H.

In both regions the daily course of temperature and humidity was thus fairly regular, and the two regions together provided a wide range of these conditions.

The wind in Somaliland also behaved fairly regularly, but in the Sudan it did not. In Somaliland, at dawn there was usually a gentle drift of 1–3 m.p.h. from the south, presumably the off-shore breeze. As the ground warmed up, the south wind at first became stronger and more turbulent ; it then either died away or veered through west and was replaced by a breeze from between north and east. This combination of the north-east trade wind and an on-shore breeze was usually established between 8 and 10 a.m. and it often reached a speed of over 12 m.p.h. shortly after noon. After that, it usually weakened but without changing much in direction. In the Sudan, on the other hand, while there was usually little or no air movement at dawn, there was sometimes a wind of up to 7 m.p.h. and it came from almost all directions in turn. After a calm dawn, there was usually a period of an hour or so between 8 a.m. and noon when the speed was between 3 and 8 m.p.h., but the direction was unpredictable. During the afternoon, the wind tended to drop, except for periodical gusts, but it was sometimes quite strong as well as gusty. These conditions were tiresome during experiments requiring a steady wind to disperse bait ; they would materially reduce the usefulness of control measures dependent on the wind.

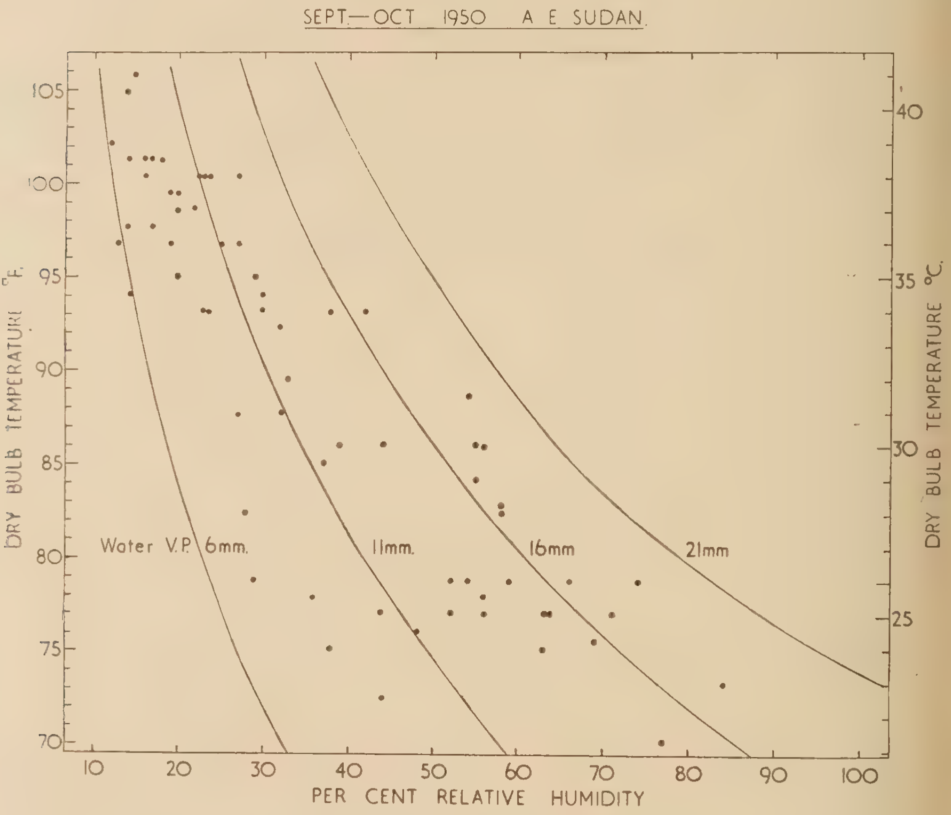


Fig. 4.—See legend to fig. 3 ; to accommodate the greater fluctuation of temperature, the scale has been reduced. The relative humidity rarely exceeded 60 per cent. and was very low in the heat of the day.

Acceptability of Bait without Water.

The world *acceptability* is used deliberately in preference to *attractiveness*, because there is little evidence in the literature that bait draws locusts from a distance. They will nibble at things they come across, even rubber insulation on electric wire ; but they will quickly eat substantial quantities of some materials, and it is such materials that are regarded as acceptable for baiting purposes.

In the initial trials, the bait being tested was laid in a continuous line, at the rate of a handful per 1–2 yards, across the front of a marching column of locust hoppers. It was easy to see when a bait was acceptable for, as the head of a column reached the line, practically every hopper would stop and feed. As soon as a few stopped, others would not as a rule readily pass them, possibly because they were mechanically impeded or because of gregarious behaviour. A few hoppers would sometimes march right across the line but, finding themselves almost alone a yard or so past the bait, on several occasions they were observed to walk back to their fellows on the bait. With a less acceptable bait, the hoppers would either march straight over it or would stop only for a moment or so before marching on. After feeding, the hoppers tended to walk to vegetation or other vertical surfaces and to perch there in post-prandial rest (Ellis, 1951).

The first tests were done with wheat bran to which the insecticide had been added in solution in mineral oil, at the rate of one imperial gallon per 100 lb. (about 10 per cent.). The tests were dramatically successful, line after line of bait becoming a seething mass of feeding hoppers as the band crowded up. It was thus clear that water was not an essential constituent of the bait and, as has been demonstrated for species of *Melanoplus* in U.S.A. (Parker, Shotwell & Morton, 1934 ; Shotwell, 1942) oiled bait was acceptable to *Schistocerca*. It was, however, tedious to mix the materials, for the oil made little difference to the texture and so it was not possible to see when mixing was adequate ; moreover, even kerosene would substantially increase the cost of the bait. Accordingly tests were done with the carrier material, both in the untreated state and mixed with insecticide in the form of dust.

On every occasion when dry bran bait was tried on dense marching bands in Somaliland (third, fourth and fifth instars), ten in all, it was fully acceptable. But, on two other occasions, when the hopper bands were not dense and the hoppers were moving thinly along a dry river bed, they hardly took the bait at all ; when the same bait was mixed with water, it was taken a little better, but still by only a proportion of the hoppers. At that place and time, there were many hoppers moulting, and those that did cross the bait lines may have been approaching the end of the instar, when both feeding and marching are much reduced (Ellis, 1951). The failure to take bait just before moulting, especially at the last moult, was observed repeatedly and was the sole common cause of baiting failures.

The successful tests in Somaliland were carried out at all hours between 9 a.m. and 6 p.m., so that, contrary to the views of the local Somalis, there was no indication of a period in the middle of the day when the locusts would not take bait. Air temperature seldom rose above 30°C., but of course the sand surface was much hotter in the continuous sunshine.

In the Sudan, dense bands marching across open ground were not available, but looser migrations of second- to fifth-instar hoppers through vegetation were found. Even in the very dry conditions there, dry bran bait was taken so well, in small rather casual trials, that comparison with wet bait seemed pointless.

There was, however, one occasion when bait failed. Between 10.30 and 11.15 a.m. one day, several bands of second and third instars were found, each extending over a few square yards. They were clustering extremely densely on the *khisheina* (probably *Indigofera semitrijuga* Forsk.), a small leguminous plant growing in an even cover of several hundred square yards. A ring of dry bran bait poisoned with benzene hexachloride was put round a band, which was not watched. When examined a little later, the locusts were still clustered, but outside the ring, indicating that a slow movement—which was also discovered on other occasions—was going on. Not more than 30 per cent. of the hoppers died, and it was evident that the bait had not been taken well. The air temperature at the time was about 35°C. and the sandy soil felt very hot to the hand. Air sucked off the sand surface with the Assmann hygrometer brought the dry thermometer to 41°C., and this certainly underestimated the temperature of the sand (Parker, 1924 ; Parry, 1951). When the hoppers were chased off the vegetation, they almost immediately perched again, hopping wildly while on the hot sand. Parker (1930) showed that *Melanoplus mexicanus* (Sauss.) climbed up off the ground when the soil surface temperature reached about 45°C., the air temperature at 4 ft. above the ground being between 22 and 31°C. An ascent of only 2 ins. generally took the grasshopper to an air temperature much nearer to that at 4 ft. than it was on the soil surface. Fraenkel (1929) reports that hoppers of *Schistocerca gregaria* march at soil temperatures of 27–40°C. and then perch at 40–50°C.

Subject to limitations imposed by moulting and by hot ground, it was concluded that bran bait without water was generally acceptable even in the dry conditions of the Sudan. When dry bait was not accepted because of moulting, wet bait was not sufficiently acceptable to justify baiting at all. When the ground was too hot for the locusts to stay on it, they would no doubt stay longer on wet bait if only because it would be cooler ; but in such dry and hot conditions, wet bait would remain wet for only a few minutes in any case (Hussein, 1950). It is shown below that when the same dry bait is scattered very thinly by machine amongst the food-plant, *khisheina*, quite complete mortality can be produced. It is therefore evident that even competition between the normal food and the thinly scattered dry bran does not prevent the bait being effective. In fact, no circumstances were found in which wet bait should be used instead of dry ; such circumstances may well exist and a watch should be kept for conditions in which wet bait is completely effective and dry bait is not.

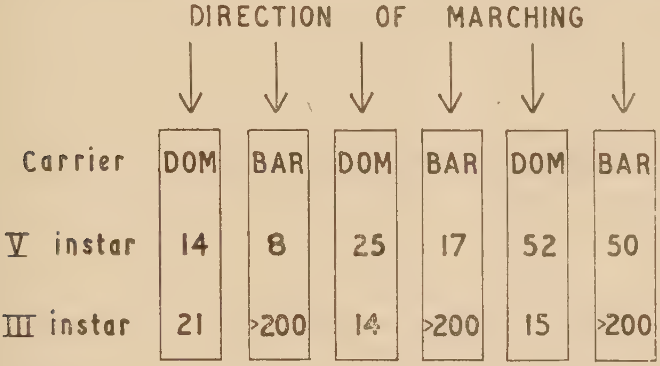
Acceptability of Carriers other than Bran.

Wheat bran is a useful food for domestic animals ; moreover, during a widespread Desert Locust plague, the demand for it for locust bait runs into thousands of tons a year and it becomes difficult to get (Notley, 1946). Consequently some simple experiments were done in Somaliland on the acceptability of other carriers, namely ground barley husks, peanut flour, dom flour, three grades of ground maize (coarse, medium and fine), and three grades of ground dura (*Sorghum* millet). The dom flour was understood to be a by-product of the manufacture of buttons from the nuts of the dom palm (*Hyphaene thebaica*).

In the first trial, a continuous line was laid in front of a column of locusts marching along a dry sandy river bed, each carrier constituting a 2-yard length of it ; all were dry and without poison. The ground grains and the dom stopped the hoppers completely, actually before they reached the line itself, where the wind had blown floury material towards the column. Peanut flour and barley husks stopped some but not all of the hoppers ; forty minutes later, there were hoppers feeding on all the carriers except these two. In a second trial, the same carriers again showed up badly, with peanut flour worst, so this was abandoned. A third trial was intended to compare the barley husks and coarse ground maize ; two successive lines were laid, half of each consisting of one of the carriers and half of the other, laid so that a hopper which marched across the husks would at once come to the maize and *vice versa*. As it turned out, both lines were so completely effective as to give no indication of relative acceptability.

It was therefore decided to try to take the edge off the locusts' appetite in the hope that they would then be more discriminating. Peanut flour was scattered thinly and widely between the hopper band and the next test lines, and this did stop the head of the column. When they moved on, both barley husks and dom flour produced a complete cessation of marching again. It was therefore evident that barley husks could be completely acceptable on some occasions and yet less acceptable than the other materials on other occasions.

During this last trial, it looked as if there was an instar preference, for the band consisted of mixed third and fifth instars, and there seemed to be more third on the barley husks and more fifth on the dom. Accordingly, six lines of alternating carrier were laid parallel to the line of march and so close together that the usual jostling would tend to move hoppers from one line to the next. Fig. 5 shows the distribution of the two instars on the two carriers Every line of barley husks was densely crowded with third and carried fewer fifth instars than the neighbouring lines of dom, which carried few third instars. Although fifth-instar hoppers are much larger than third, the numbers do not suggest that the third instars were jostled off the more acceptable bait. The result was the more surprising because the previous experiments had ranked dom high and barley husks low for both instars. It seemed unlikely that this sort of inconsistency could be resolved into an order of merit in the few days available, so attention was concentrated on the two materials likely to be available cheaply.



Carrier	DOM	BAR	DOM	BAR	DOM	BAR
V instar	14	8	25	17	52	50
III instar	21	>200	14	>200	15	>200

Fig. 5.—Food preferences apparently differ at different instars, fifth instar preferring dom flour and third instar barley husks.

Barley husks and dom flour were tried both dry and poisoned with toxaphene in oil to a strength of 1½ per cent. and compared with dry bran. The tests, which were with fourth-instar hoppers, were quite unfavourable to the barley husks. Even if this material is acceptable to third instar, it was so unacceptable to fourth and fifth that it cannot be recommended at all. On the other hand, dom flour, whether plain or mixed with toxaphene solution in oil, was generally acceptable ; there was just one occasion when hoppers which had either fed on dom, or possibly marched across it, immediately stopped on plain bran. This result may not have been due to inferior acceptability of the dom flour and this material is worth persevering with.

Although each of the carriers was eaten to some extent by the hoppers, some of them were passed over by hoppers on occasions when other carriers were well taken. It is clearly undesirable to use a bait which will be sporadic in its effectiveness if better material is available. The use of peanut flour and barley husks is therefore not recommended, dom flour could well be used instead of wheat bran, while the ground maize and dura seemed quite satisfactory on the few occasions when they were tried.

Line Baiting by Hand.

Placing a continuous line or several lines of bait across the path of marching locusts is quite the most economical and effective method of locust control. The bait can be laid sufficiently thickly to ensure that the hoppers are bound to touch it ; and if the hoppers are marching well, there is reason to believe they will feed, for the ground is evidently not too hot for them to stay on it and they are evidently not just about to moult (Ellis, 1951). With less certainty but frequent success, bait lines can profitably be laid around roosting hoppers or bands of hatchlings. There were four experiments in Somaliland where the areas of the hopper bands and the amounts of bait used for clearance were estimated ; in three of them the areas were between 2 and 12 acres and the amounts of dry bran bait used in the lines averaged 20 lb. / acre or less. At current prices, with 0·5 per cent. BHC, this would cost under 3s. per acre for material. The fourth was only 0·2 acre and the dosage twice as great.

Now that dry bait can be used for this purpose, there is no reason why locust scouts should not, when searching for hoppers, deal with the smaller infestations in this way. It is quite easy for a man with a camel, a donkey, or a vehicle, to take a sack or two of bait and to ring bait or line bait without help, so that only large infestations need be reported for action by control teams. It is therefore worth while to improve the details of the present technique.

In many areas, the standard container for bait that is being scattered is a 4-gallon petrol tin with the top cut out. Such a watertight container with firm walls is necessary for wetting bait but is inconvenient for dry bait. The dry bait is easily scattered by a light wind so, in laying a line, the operator has to stoop to bring his hand near the ground ; the tin then tends to get in the way or to empty, if it is tucked under the arm. The 7-lb. bags in use in the Sudan are quite convenient, and an even simpler method, suggested by Mr. A. S. McKinnon of British Somaliland, makes use of an empty sack. At one end of the sack, a 2-foot length of string is tied to each of the two corners, and the two strings are tied round the waist, leaving the sack hanging like an apron. The other two corners are gathered into the left hand and about 10 lb. of dry bait can be poured into the bag thus made. The apron-bag is comfortable to wear and does not spill in use. It is easy and simple to lay a continuous line, throwing forwards a handful each time the left leg goes forward and filling the hand at the alternate pace. Somalis were quickly trained in this technique of line baiting and were very successful in unsupervised baiting against marching bands ; they evidently enjoyed planning where to lay the lines of bait to catch all the columns of locusts without disturbing their march and they appreciated omitting the laborious mixing of bait with water.

Line Baiting by Shendi Funnel.

Dry bait flows so much more freely than wet that machine handling becomes relatively easy, and some sort of funnel is an obvious means of laying an even line from a vehicle. After trials of various shapes and sizes, a simple and workable design (fig. 6) was decided upon and named the Shendi Funnel, after the home-town of my colleague, Haider Effendi, and the place where it was made by the local tinsmith. The essential features of the design are that the cone should be steep, with a slope not more than 25 degrees to the vertical, and that there should be no ledges on which the bait can catch and begin to build up. If it does build up, it quickly forms a hollow roof over the exit ; in case this happens, a piece of stout wire is loosely fixed so that it can be moved by hand to break the cave. Using opened-out petrol tins, the funnel was easily made in an hour or so, with swivelling loops for roping to the back of a vehicle.

With dry bran the funnel shown in fig. 6 has an output of about 10 lb. per minute ; with bait which had been made up by adding arsenic solution to bran and then drying (King, 1929), which was denser, the rate was about 20 lb. per minute. A line consisting of a handful of bran per 2 yards works out at a pound per 10 yards, so that a vehicle will have to travel at about 3 m.p.h. for the dry bran and 7 m.p.h. for the arsenic bran to lay about the same dosage. The funnel lays the bait more evenly than can be done by hand and a speed several times as great could be used for small bands of hoppers. For producing a narrow but dense line of bait, the delivery pipe of the funnel should be near the ground, and this arrangement may be best for ensuring that a marching band stops on the bait. To produce a wider but less dense line, on which a larger number of hoppers can feed at one time, the funnel could be slung higher up and in such a position that the bait is caught and scattered by air movements before reaching the ground.

By this technique, a small band of hoppers was ring-baited in the Sudan in a fraction of a minute without stopping the vehicle. Once bait is in the funnel, it

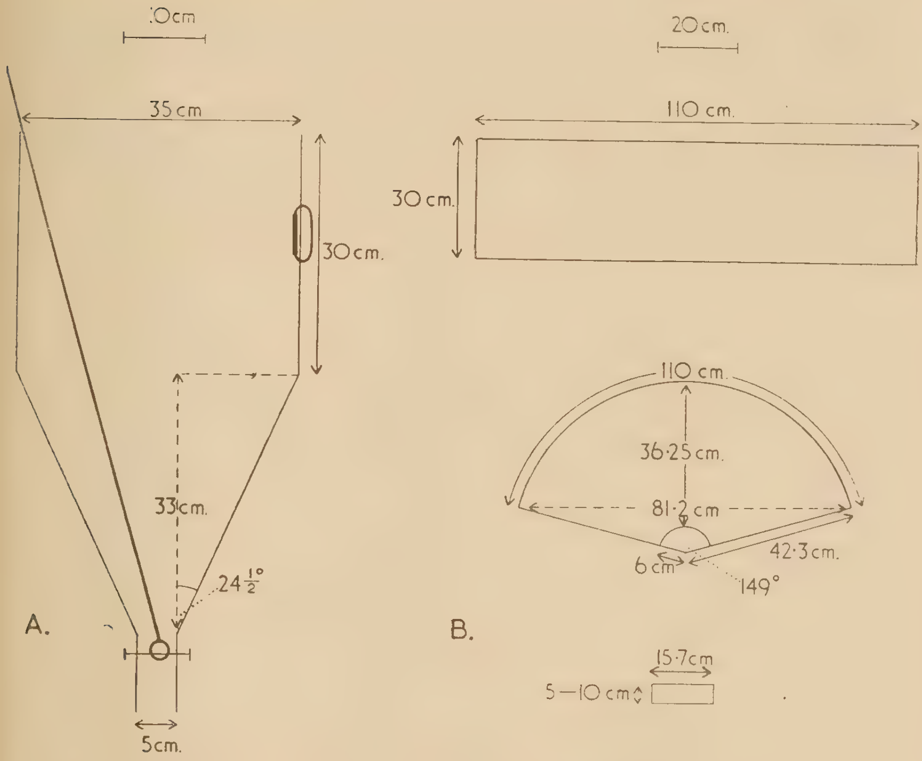


Fig. 6.—Shendi Funnel, to scale. A—in section. The half angle of the cone should not exceed 25 degrees. B—dimensions of sheet metal required. The measurements do not allow for overlap at joints. To convert centimetres to inches, divide by five and multiply by two ; the result will be only 1·6 per cent. too high.

will run out even if the vehicle stops so, to avoid leaving a little pile of bait, which may be dangerous to stock, the funnel should be allowed to empty itself before stopping the vehicle.

Area Baiting by Dusting Machine.

When the locusts are thinly and widely dispersed and are not moving much, line baiting cannot be effective. It is probably not feasible to spread bait at all evenly by hand at much less than 100 lb. /acre, so that with a thin population the material and labour required per locust are very high. Good control of low populations of range grasshoppers has been obtained in the United States by dosages of 5 lb. /acre of oiled bait. Consequently tests were made of area baiting with dry bran bait.

A motor dusting machine (Messrs. Drake & Fletcher's " Dustejecta "), equipped with a 6-foot vertical extension pipe intended for dusting trees, was mounted on a Land Rover. The vehicle was run across the wind (velocity about 11 m.p.h.) and the dust-bin emptied its 15 lb. of bran in 45 seconds. Inspection of the ground revealed no bran for the first 10 yards, a deposit which was easily seen and reasonably uniform for the next 30 yards, and a detectable deposit for the next 10 yards. The fine floury material could be seen passing overhead at 100 yards down wind from the vehicle and it was the flaky particles which floated well but descended soon enough that gave the 30–40-yard visible swath. The only further experiment done in Somaliland expended dry BHC-bran bait at about 11 lb. /acre on 5 acres infested at about five hoppers per square yard. The mortality appeared to be between 50 and 75 per cent. in 24 hours, but its estimation was complicated by population movement. The idea was worth following up.

In the Sudan, six experiments were done with dry bran bait, half of them using arsenic and half with BHC. Table I details the quantities. It was difficult to control the rate of output of the bait, for it has to be continuously stirred with a stick. A dust-bin designed like the Shendi Funnel (p. 682) would have been much better. The mean rates of emission were 32–42 lb. /min. for the arsenic bait and 23–29 lb. /min. for the BHC bait, while moment to moment variations were probably much greater. Consequently, the area dosages applied were not those intended, nor were the deposits uniform. As was expected with a 6-m.p.h. wind, there were, within the 50-yard swaths, strips on which no bait could be seen, especially with the arsenic bait.

In view of this unevenness, it was surprising that in three of the experiments mortality was virtually complete (marked ++ in Table I). In these three and in the fourth against hoppers, the vegetation was mainly *khisheina*, which did not leave any large or continuous areas of bare ground ; the young seed pods of this plant were eaten by the hoppers. The other two experiments were done against adults in dura (*Sorghum* millet) at all stages of growth from a few inches high to the milk stage of the grains. There was plenty of bare ground between the plants, where the bait could be seen and where adults did sit at times. The 3-ton truck on which the dusting machine was mounted damaged considerably less than 1 per cent. of the plants. Two common features of the experiments with incomplete mortality were the wide swath and the low dosage. If the machine, methods, and baits, are not to be changed, the following quantities would probably be appropriate for routine control : area dosage, 25 lb. /acre ; swath width, 35 yards ; the truck speeds required would therefore be about 7 m.p.h. for arsenic bait and just under 5 m.p.h. for BHC bait. Owing to the less flaky nature of the arsenic bait, which had been wetted during preparation (King, 1929), it fell to the ground more quickly than the dry-mixed BHC bait ; it might therefore be desirable to decrease the swath width for arsenic bait to 25 yards and correspondingly increase the truck speed to 10 m.p.h. This is a high speed at which to operate unless the ground is very level and clear.

TABLE I.

Dry bait distribution with motor dusting machine.

Expt.	Date 1950	Locust instar	Poison	Wind speed m.p.h.	Area acres	Dosage expended lb./acre	Swath yards	Speed acres/ min.	Mortality
5a	15/9	Adult	BHC	3–5	55	12	50	1·2	?
5b	15/9	Adult	arsenic	3–5	65	12	50	1·8	?
11	30/9	III	arsenic	5–8	26	46	25	0·7	++
14	3/10	IV	BHC	6–9	17	55	25	0·4	++
16	5/10	IV	BHC	5	15	8	50	2·2	+
20	9/10	V	arsenic	5–12	18	30	25	0·6	++

++ indicates virtually complete mortality.

Apart from improving the feed of the dusting machine, mainly by using a steeply conical dust-bin and increasing the height of the emission pipe as much as possible, improvements might be made in the bait. It was quite clear that the floury part of the bran floated away on the wind and was lost and it was mainly the flakes, which floated sufficiently but not too far, that formed the swaths. Mr. Joyce has therefore suggested arranging for the component materials to be separated out, either in milling or subsequently, and using the flour for other purposes (Joyce, 1952).

The area dosages required seem rather large, compared with North American practice ; according to samples very kindly sent by Mr. Claude Wakeland from Denver, Colorado, U.S.A., a much more flaky bran free from flour is used there, so that the additional dosage we had to use may have been simply flour which was lost on the wind and not deposited within the target area at all. With the insecticide mixed in as a dust, the flour, with its large specific surface, might well have carried an undue proportion of the insecticide. If machine methods of distributing dry bran bait became widely adopted, the composition of the bait could well be a profitable subject of investigation, and it might turn out that different compositions would be preferable for different purposes.

Labour.

There are too many variable items in locust control to justify making close comparisons yet of the costs of various techniques ; but the choice of methods, which will usually be mainly made so as to produce an effective campaign, may sometimes be assisted by considerations of cost. Costs are frequently inflated by the amount and inefficiency of local casual labour required, so a slight consideration of this may be worth while.

According to Cañizo (1941), in Spain six men can distribute a ton of bait per day, an average of about 350 lb./man-day. In Somaliland a test was done to estimate the the amount of labour used in hand dry baiting. The area was reasonably flat and clear of trees, and therefore ideal for supervision. The area dosage was about 100 lb./acre. Counting the time taken to assemble the men on the baiting line and including baiters, headmen, cooks, truck crew, and so on, on the average it took one man one day to bait one acre under European supervision.

A comparison was made between dry baiting and wet baiting, under conditions which produced a considerably higher rate for about an hour. The interesting feature was that it took two men to mix enough bait with water to keep one man occupied in spreading it. Taking account of the extra handling and milling around, wet baiting required four times as much labour as dry baiting. It is almost inconceivable that it would be a day's work for one man to wet-bait only ¼ acre, but this may well be above the average achieved by widely scattered gangs with little skilled supervision.

With machine dry baiting, an average of ½ acre per minute, including turns, should not be difficult to achieve. The men required for this are : a truck driver, a mechanic to operate the dusting machine and stir the bait, a boy to open the sacks, and a man to load the dust-bin almost continuously from the sacks, two men to measure out the swath widths, and two boys to hold flags to guide the driver. When the truck approaches the end of a line, both the measurer and the flag boy must act as markers, so that the next line can be started without delay. Including a supervisor, who should be reliable, this makes a total of nine, of whom three would be paid much more highly than casual labourers. The same truck can carry a ton of bait, so a self-contained unit could cover 90 acres in 3 hours, and even if the rest of the day were spent in travelling to and from base and between target areas, those nine men with truck and machine could do the work of at least 90 hand baiters. There are too many variables involved to make it worth while to pursue the comparison further.

Baiting Adults.

The first requirement in locust baiting is to establish contact between the locust and the bait. The most obviously successful operations were those on marching hoppers, when contact was established quickly and completely ; the least successful ones, leaving aside hoppers about to moult and cases where the ground was too hot for the locusts to remain on it, were attempts to kill adults. In the Anti-Locust

Research Centre laboratory, especially shortly after the last moult, adults eat considerable amounts of dry bran, though less than V instar hoppers. In Somaliland, there was not a scrap of evidence that adults, once touching bran bait, ignored or rejected it ; on the contrary, fledglings marching with hoppers took dry bait and died like the hoppers. On at least five occasions, lines of bait which had been laid for marching bands were seen several days later and found to be dotted with corpses of adults and with live adults feeding. On the other hand, deliberate attempts to destroy young adults were only partly successful. In area baiting, they flew off as the baiters approached ; when bait was laid around trees where they were roosting, many adults were killed, but mortality was never complete. Some, but not all, of the locusts did bask on the ground in the morning, and it seems likely that the failures were simply due to failure to establish contact between locust and bait. When adults can be depended upon to come to the ground, as when egg-laying, they should be baited ; this has already been successfully done with wet bait (Rungs, 1946).

In the machine baiting at 12 lb./acre against adults in dura in the Sudan, it was impossible to estimate mortality, but it was certainly not complete. In this case, too, failure may have been due to the same cause, either because too few of the locusts came to the ground, or because there was not enough bait there when they did.

Insecticides.

In careful field trials against species of *Melanoplus* in the United States, it seldom happens that mortalities close to 100 per cent. are claimed and the usual figure is between 70 and 80 per cent. This may be satisfactory for a relatively non-migratory grasshopper in reducing damage below the level of economic importance, though the efforts made to improve baits suggest that it is not satisfactory. For the Desert Locust, such a low mortality is almost useless, for the survivors would still form swarms and migrate into other regions, thus necessitating campaigns elsewhere ; techniques which do not usually give 100 per cent. mortality are therefore most undesirable. The insecticide in bait costs less than the bran, usually far less, and it is certainly responsible for a small proportion of the total cost of a campaign. It would therefore be unwise, to say the least, to economise on insecticide if that were responsible for a failure to reach the 100 per cent. mortality.

Mortalities very close to 100 per cent. were certainly achieved in some of the trials in the Sudan. The terrain was sometimes very favourable for demonstrating this, for it consisted of many acres of continuous *khisheina* on sandy ground, broken only by occasional tufts of a grass of the growth habit of *Panicum turgidum*. When the Land Rover was driven through this, it left a track which was visible from the side for 5 yards or so. If any hoppers survive, they can be detected by driving the vehicle in a spiral from the central point, spacing at about 5 yards from the previous turn. The driver and two passengers concentrated their attention on insect movements close to the vehicle and it was shown that a single hopper could be repeatedly found when, by accident or design, the same track was repeated. The spiral track was continued until an area had been covered out of which migration could hardly have taken place.

By this technique, when 46 lb./acre of arsenic bait (2·5 per cent. sodium arsenite) were supplied by machine, within 36 hours less than 10 survivors were found from several small bands totalling not less than 5,000 hoppers. A similar result was obtained with 0·5 per cent. BHC in a shorter time. It is not at all clear why such a favourable result should be obtained, compared with those obtained by American workers ; it may be that the ages of our hoppers were so uniform that there was no failure due to the approach of moulting (Langford, 1930 ; Common, 1948 ; Husain, Mathur & Roonwal, 1949), or that the nature of the terrain or vegetation were more favourable.

With low dosage dry baiting, two properties of the bait are worth special consideration, namely the effectiveness of a small amount of bait, and the persistence of its toxicity. When the bait is very thinly scattered, random contact between the locust and a flake of bait may be established only rarely. The ideal is therefore that every single flake of bait should be enough to kill, and that its power to kill should be retained for long enough to ensure destruction of the full 100 per cent. of the population, if that is fairly static. To achieve this, the bait should be designed so that the average concentration of insecticide is adequate and it should be made up so that mixing is really effective. There was evidence from line baiting in Somaliland that some locusts which had fed did not die, which casts suspicion on the adequacy of mixing.

As to the persistence of the bait, when it is applied dry it remains acceptable for days or weeks. In the machine dry baiting in dura against adults in the Sudan, where the population was relatively static for weeks, death from BHC ceased after about 3 days ; death from arsenic did not begin until the following day, but it went on for at least 10 days, after which heavy rain fell. The BHC certainly loses its effectiveness long before the arsenic. Although arsenic has the disadvantage, which is often overriding, of high mammalian toxicity, its application by machine in low dosage could be justified in some cases where prolonged toxicity to locusts would be valuable. This would be especially the case with a thin static population during a prolonged dry season, such as is experienced with Red Locusts in the Rukwa Valley, and a test of the method seems desirable there.

Discussion.

The development of poisoned bait for killing locusts and grasshoppers has, in recent decades, been largely a process of simplification. Molasses, banana oil (amyl acetate), and salt, which had been thought to be attractive, are not now used. In North America, the successful use of oiled bait has shown that even water is not always essential, while in the U.S.S.R. dry bait has been recommended for use especially for aircraft dissemination (Beĭ-Bienko, 1934).

Dry baiting against the Desert Locust is not a new thing ; to my knowledge it had been effectively tried by Beshir effendi Babiker el Shafie in Arabia, by Mohammed Bey Hussein in Egypt (Hussein, 1950) and by Messrs F. J. D. Thomas and J. F. Graham in Kenya. But it had never been taken up as the routine method in preference to wet baiting. There is now no reason to continue wet baiting, but a careful watch should be kept for circumstances in which dry bait fails and wet bait is quite successful.

Bait wetting must be done just before scattering or it becomes unacceptable. The lack of deterioration of the carrier in dry bait, both before and after scattering, allows much greater flexibility in use. Every scout can carry some and can clear up small infestations without delay ; the effectiveness of the bait, once laid, persists as long as the insecticide, or until it is blown or washed away.

Dry bait flows to some extent, while wet bait does so hardly at all. With a carefully designed funnel, dry bait can be made to flow well for line baiting. It has to be stirred to flow well enough from the dust-bin of the ordinary dusting machine, and a dust-bin shaped like the Shendi Funnel is required.

Further trials on the acceptability of dry bait need only be done if it is found not to work in routine control on occasions when wet bait does work ; as precise as possible a description of the circumstances would be necessary before further trials would be worth starting. An investigation of the properties of bait carriers, which make them acceptable, may be profitable, and preliminary work on that by Miss P. M. Davey has begun in the Anti-Locust Research Centre. Immediate field trials are also likely to be valuable. To that could be added an investigation of the concentrations of insecticides required, especially for low dosage area baiting, with emphasis on insecticides which are relatively harmless to mammals and also very persistent. It seems likely that the Shendi Funnel would be a useful instrument as standard equipment for all motorised scouts and for mobile emergency units. Developments in the use of the dusting machine are likely to arise from control operations, if that is found to be a convenient technique.

This recommendation to use dry bait against the Desert Locust may well cause surprise and it is indeed surprising that in very dry hot air this locust will take dry bait, even when the natural food is growing all around. It is not contended, however, that the dry material is in any way preferred either to the natural food or to wet bait. Indeed, it has been pointed out that when bait was not readily acceptable, more hoppers took wet bait than dry. A recent publication by Smit (1951) describes carefully designed and analysed field trials against the Brown Locust (*Locustana pardalina* (Wlk.)) in which dry BHC maize bran gave an average mortality of 53 per cent. and the same bait wetted gave 77 per cent. The conditions of trial were, however, very severe. The estimates of mortality were based on caged samples and, in order to avoid bad sampling due to some of the insects being already immobilised, catching began as soon as the first affected hopper was noticed. Since immobilisation sometimes occurred within 5 minutes of taking the BHC bait, and some individuals would presumably take bait as soon as it was scattered, the time allowed for the most sluggish to feed would be very short. Such an arrangement is calculated to show up slight differences of acceptability when a quick-acting poison is used. Smit did not test dry bait containing the slower-acting poison, sodium arsenite, with which the same technique of test would have allowed a much longer time for feeding to begin. In control operations there is no such limitation of time, and, as De Wet (1941) has pointed out, Brown Locusts will eat bait that has lain long enough after scattering to dry up. Using arsenic-bran baits, Paul (1942) could find no significant deficiency in mortality of *Melanoplus mexicanus* and *Camnula pellucida* in Canada when dry bait was used instead of wet.

Summary.

Field trials in British Somaliland and in the Anglo-Egyptian Sudan showed that dry bran bait can be used for complete destruction of Desert Locust hoppers (*Schistocerca gregaria*). Wet bait was never found to be completely effective when dry bait was ineffective.

Failures of dry baiting were found which were attributable to several causes : hoppers not feeding just before moulting ; ground too hot for hoppers to remain on it ; too low or too patchy a deposit of bait ; adult locusts probably not making contact with bait. None of these failures could be attributed to the bait being dry.

The main constituent of standard bait, wheat bran, should not be replaced by barley husks or peanut flour, but dom flour could be used and dura and maize flours could be tried instead of bran.

The use of dry bait makes it convenient and practical for locust scouts who use camels, vehicles, etc. to destroy small bands of hoppers as soon as they find them.

Dry baiting against marching bands and ring baiting can be done very conveniently and speedily by means of the Shendi Funnel, designed to be tied to the back of a vehicle.

Area baiting can be done rapidly by blowing dry bait up from a dusting machine, so that the wind spreads it. An area-dosage of 25 lb./acre is recommended, and a rate of $\frac{1}{2}$-acre/minute should be readily achieved.

The amount of labour required is reduced to about 25% by the use of dry bait instead of wet. Dry baiting by machine uses only 10 per cent. of the labour required

for dry-baiting by hand, but the cost would not fall as much as this, because higher wages would have to be paid for the greater skill necessary.

Adults can be killed by baiting, but it is much more difficult than with hoppers to ensure that the locusts make contact with the bait.

The insecticides already in use against the Desert Locust, arsenic and benzene hexachloride, can completely destroy hoppers when applied in bait. An insecticide is required with a long persistence like that of arsenic and a low mammalian toxicity like that of BHC.

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FIELD TRIALS WITH VARIOUS DRY BAITS AGAINST THE DESERT LOCUST, *SCHISTOCERCA GREGARIA* (FORSK.).

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As a result of trials in the Shendi District of the Sudan, dry bran bait, instead of wet, has been recommended for use against the Desert Locust, *Schistocerca gregaria* (Forsk.) (Gunn, 1952). During the winter campaign 1950–51 on the Red Sea Littoral, both arsenic (2·5 per cent. sodium arsenite) and BHC (0·5 benzene hexachloride) bran baits were used entirely without water, except at Tokar Delta, where the strong winds blow dry bait away. No cases of failure were reported, and supervisors were enthusiastic about the savings and the results. The initial success was gained against a large invading swarm of adults during November, as a result of which hatchings were light and practically all the hoppers were cleared by dry baiting before they reached the third instar.

Accepting, therefore, the efficacy of dry bran bait, there remained a problem of obtaining enough of a suitable alternative carrier material. Wheaten bran had become difficult and expensive to buy and efficient substitutes were urgently required. Two materials were available : South African surplus stocks of maize meal and maize bran containing 2·5 per cent. sodium arsenite, which were cheap and abundant ; and crushed dura (*Sorghum* millet) or maize, which were considerably cheaper than wheaten bran. Both materials were promising, for Mr. J. F. D. Thomas (private communication, 1946) had found dry maize to be so in Kenya, while dry crushed dura

TABLE I.

Daily meteorological records, all made at Wadi Coreab except the first two pairs, which were at Mohammed Qol and at Agwer, respectively.

Date Dec. 1950	Time	R.H. %	Temp. °C.	Wind		Cloud	
				Est. speed m.p.h.	Direction deg.	Type	Sky covered
23rd ...	08.00	61	28·5	15	195	Cu.	<1/10
	12.00	61	30·0	25	152	Cu.	<1/10
	21.00	92	23·5	10	165	—	0
24th ...	08.00	83	22·0	7	125	—	0
	19.00	84	24·5	20	170	—	0
25th ...	08.00	91	21·5	2	155	Cu.	<1/10
	12.00	61	30·0	15	*ca.* 5	—	0
	17.00	84	24·0	10	152	—	0
26th ...	08.00	91	19·5	1	155	—	0
	12.00	50	30·0	5–15	110	Cu.	<1/10
	17.00	77	25·0	0	—	(Heavy over hills)	
27th ...	07·00	91	21·0	0	—	Cu.	<1/10
	10.00	61	30·5	5	135	—	0
	18.00	100	22·0	10	120	Heavy *	8/10
28th ...	08.00	91	21·5	1	135	Heavy	7/10
	13.0	61	30·0	10	135	Cu.	<1/10
	17.00	70	25·5	15	130	Cu.	1/10
29th ...	06.00	91	21·5	2	170	Horizon	<1/10
	12.00	61	31·5	10	145	—	0

*Trace of rain.

had been found to be actually more acceptable to grasshoppers in the central rainlands of the Sudan than wet wheaten bran (Joyce, 1952).

The objects of the work now reported were therefore to assess the acceptability of maize and dura baits, and to find the most suitable particle sizes.

TABLE II.

Percentages of samples of carrier materials stopped by various sieves.

Bait	Sieve : meshes per inch				Note
	10	20	30	40–100	
Wheaten bran	Trace	29	29	42	Local (Sudan).
Dura coarse ground ...	Trace	67	10	23	Variety Wad Aker.
Dura medium ground ...	1	41	17	41	Vareity Feterita.
Dura fine ground	Trace	Trace	2	98	Variety Feterita.
Maize fine ground	Trace	12	37	51	Local (Sudan).

Weather and Vegetation.

The topography and vegetation of the Red Sea Littoral has been described by Maxwell-Darling (1936). The present trials were conducted near Halaib some 200 miles north of Port Sudan, between the foot-hills and the belt of salt-bushes. Topographically the area is a pebbly plain, supporting little perennial vegetation, dissected by wadis and smaller water channels in which *Panicum turgidum* Forsk., *Acacia raddiana* Savi and *Lycium persicum* Miers were common. The rains had been good and generally a good cover of annuals was present, varying in density from not more than 12 on parts of the pebbly plain, to as many as 1,000 plants per square metre, of which *Aristida* prob. *funiculata* Trim. & Rupr., *Aristida* sp., *Chloris* sp. prob. *virgata* Swartz., *Eragrostis ciliaris* Lint. (Graminaceae), *Crotalaria* sp., *Tephrosia* sp. (Papilionaceae), *Portulaca* sp. (Portulaceae), and *Polygala erioptera* D.C. (Polygalaceae) were the most common. None of these plants grew to a height of more than 50 cm. The food plants which appeared to be most favoured were flowers and seeds of *Panicum turgidum*, and the flowers of two annuals which have not been identified.

During the trials, between 23rd and 29th December, the weather was generally cool ; air temperatures rose from 20–22°C. up to 8 a.m. to about 30°C. by noon, falling by 5–8° by 6 p.m. Air humidity was around 60 per cent. at noon and, of course, higher at the lower temperatures. Wind was generally southerly (110° to 195° Mag.). Table I gives the daily meteorological records.

Materials.

Samples of the carrier materials available in the Sudan were sieved and the distribution of sizes is shown in Table II. From this it will be seen that 29 per cent. of the wheaten bran was stopped by a 20-mesh sieve and a further 29 per cent. was stopped at 30-mesh. Over 40 per cent. of the sample passed through the 40-mesh and it was therefore a floury bran.

Table III shows a chemical analysis. Sieving the Feterita dura seemed to leave slightly more protein in the larger particles and the maize contained larger quantities of fat, but otherwise the materials were chemically very similar. The larger particles in the coarse dura were not simply bran, but contained pieces of broken grain left by the crushing process. In the experiments, unsieved materials were sometimes used and sometimes sieved fractions.

The locusts were first, second and third instars, mostly in small patches of no more than 50 sq. yds. Marching and feeding generally continued throughout the day. The direction of march was remarkably constant, with the wind and so towards north and north-west. One band of second instar hoppers covered 2 km. in one day and an observer had to be detailed to keep track of each treated band.

TABLE III.

Chemical analysis of some bait carriers.

Material	Moisture %	Ash %	Protein %	Fat %	Fibre %	Carbohydrate %
(1) Maize 30 mesh ...	5·22	2·40	10·21	3·64	0·86	77·67
(2) Wad Aker 30 mesh ...	5·02	1·85	11·45	2·15	0·64	78·89
(3) Feterita bran 10–30 mesh	4·67	2·12	12·12	1·38	0·60	79·11
(4) Feterita 40 mesh ...	4·65	2·01	10·29	1·72	0·73	80·60

Methods.

Acceptability was estimated directly only on bands of marching hoppers (Gunn, 1952). If a line of acceptable bait is laid squarely across the path of a band, as the hoppers reach the bait they halt, unanimously start feeding, and continue feeding for some time. If the bait is less acceptable, some do not stop at all, and others do not stay very long. Other things being suitable, the number of hoppers feeding therefore gives an indication of acceptability. If several baits are placed in a series forming segments of the line, an indication of their relative acceptability can be obtained from the numbers feeding. It should be understood that in this test few if any hoppers make contact with more than one bait, so that there is no question of their choosing between baits. They come to the one bait which happens to be in their path, and they take it to one extent or another, or they leave it.

In using this technique, the segments of the bait line were made up of trays 20×12·5 cm. and 15 mm. deep, sunk so that their tops were level with the ground. Each tray contained one kind of bait, and the kinds were replicated and randomised in a single line. The hoppers feeding on each tray were counted or estimated at intervals.

TABLE IV.

Expt. 1.—Numbers of hoppers counted on trays of bait.

Poison	Carrier	Replicates—numbers of hoppers						Totals
BHC ...	Wheat bran ...	33	425	331	271	293	130	1,483
BHC ...	Dura—coarse (Wad Aker)	109	445	235	255	228	151	1,423
BHC ...	Dura—fine (Feterita)	312	370	318	226	250	145	1,621
Arsenic ...	Maize bran ...	151	330	46	34	56	15	632

The major disadvantage of this technique is that the density of hoppers varies in a band and particularly tends to fall near the sides of the column. Consequently fewer hoppers arrive at the trays at the sides of the column, and the end trays may not be in the line of march of any hoppers at all, although the baits on these trays may be very acceptable. Even well within the column, variations in density will affect the numbers feeding on the various trays, and consequently replicate trays at different positions on the bait line may give very varied results.

Acceptability can also be estimated indirectly by the mortality arising from a single type of bait. This was done with area baiting by hand. Mortality in locusts is extremely difficult to estimate and in this case was done simply visually ; a figure of less than 99 per cent. is unsatisfactory.

Tray Experiments.

Experiment 1.—There were 24 trays, made up of six replicates of four types of bait. The hoppers, third instar, marched reasonably evenly on to the line. Table IV indicates no notable difference between wheat bran and two grades of dura with BHC, but arsenic maize was significantly less acceptable (*cf.* Lea, 1949). Nevertheless mortality in the band as a whole was complete, so that even if the hoppers did not stay long on the arsenic-maize, they nevertheless stayed long enough to eat lethal amounts.

Experiment. 2.—There were 30 trays, with six replicates of five baits. The column was much narrower than the length of the bait line, so some trays were outside the line of march and were not visited. The band, composed of mixed first and second instar, stopped only briefly, and next day many of the locusts moulted, so that little food could be expected to have been eaten on the day of the experiment (Ellis, 1951). Although arsenic-maize bran again showed up worst, the differences are not significant (Table V).

TABLE V.

Expt. 2.—Total numbers of hoppers on six replicates of five baits.

Poison	Carrier	Totals of hoppers
BHC	Wheat bran	408
BHC	Dura—coarse (Wad Aker)	348
BHC	Dura—fine (Feterita) ...	405
Arsenic	Wheat bran	375
Arsenic	Maize bran	345

Experiments 3 & 4.—These experiments were not very successful because marching was poor and again many hoppers moulted on the following day.

Experiment 5.—The hoppers were freshly moulted second instar. The bait trays were laid in two parallel lines a metre apart, each line containing six baits replicated five times. The trays were not filled with bait, as previously, but each had 5 cc. (about 10 gm.) sprinkled on it. This amount on a tray of 250 sq. cm. area works out at 3,450 lb./acre and is roughly a normal rate for line baiting by hand (Gunn's hand baiting uses a ton per acre of bait-line). Compared with dosages used in area

TABLE VI.

Expts. 5 and 6.—Total numbers of hoppers on five replicates of six baits.

Poison	Carrier	Totals of Hoppers	
		Expt. 5	Expt. 6
BHC ...	Wheat bran	2,240	1,802
BHC ...	Dura—coarse>20 ...	1,845	1,465
BHC ...	Dura—medium>30 ...	2,569	2,285
BHC ...	Dura—bran	2,160	2,184
BHC ...	Dura—fine<30 ...	1,911	1,650
Arsenic ...	Maize bran	825	—
BHC ...	Maize crushed	—	1,866

baiting, this appears very high, but to get a comparable figure, the dosage should be related to the length of marching column of locusts which feeds off the line. The bait was almost completely eaten up and the mortality was over 99 per cent. The figures for the number of hoppers on each bait are summarised on Table VI, column 3.

There was very great variation between the replicates, so that only the arsenic-maize bran can be said to be inferior in acceptability although again it must have been quite satisfactory for control purposes, since mortality in the bands as a whole was complete. Clearly the various grades of dura do not show inferior acceptability even to wheaten bran.

Experiment 6.—The hoppers were again second instar and the layout was the same as in Experiment 5. Table VI, column 4, shows the results. Crushed maize with BHC was not inferior to the dura and wheat bran. Of the other baits, it is notable that medium dura scored the highest in both experiments, and coarse dura the lowest.

General results.—The results of the tray experiments have been examined statistically, but replicates gave wide-ranging figures and only one of the differences is significant—namely the reduced numbers on arsenic-maize bran. The work could not be elaborated to assign a cause to this, but it is noteworthy that there was no deficiency in overall mortality when arsenic-maize bran was used.

Although the other differences were statistically insignificant, it is worth noticing that the following order of acceptability of BHC baits is consistent with most of the results, the three central items being almost interchangeable.

Dura-medium (20–30-mesh)
{ Dura bran
{ Wheaten bran
{ Dura–fine (passing 30-mesh)
Dura–coarse (20-mesh)

In the single experiment with crushed maize (expt. 6), that carrier occupied a middle position in the list. Clearly, therefore, these experiments provide no reason whatever for using wheaten bran instead of dura or maize products, a very satisfactory result in view of the relative availabilities of these materials. If anything, they direct attention to particle size as an important property in determining acceptability. The medium and bran fractions may be somewhat more acceptable ; they are certainly less likely to be blown right away by the wind, and they are not so valuable for human food as the flour, so it may be worth while to fractionate crushed dura and make the best economic use of each fraction.

Mortality Trials.

Altogether ten line-baiting trials were done with a single type of bait each and two trials with more than one type ; as it turned out, these two provided no extra information, so all 12 may be considered together.

In six trials, the 24-hour mortality was low and many of the hoppers moulted on the following day, which adequately explains the low mortality (Ellis, 1951) ; but the hoppers in three of these cases then marched on to the bait and the 48-hour mortality was over 90 per cent. The baits were either fine dura or wheat bran, with 5 per cent. of 20 per cent. BHC, *i.e.*, 1 per cent. BHC, or about 0·13 per cent. gamma BHC.

In four trials, using 0·5 per cent. BHC, with fine dura (three trials) or wheat bran (one trial), the mortality was equally high in 24 hours. It was an unfortunate coincidence that the higher dosage of poison was given to bands that moulted and the lower to bands that did not, so no conclusion could be reached about the better dosage to use. In any case, the estimates of mortality were made simply by inspection.

The other two trials offered several baits in parallel lines, but the first line caused complete stoppage, and once more the mortality was over 90 per cent. It may well be valuable, however, to lay several successive lines of bait, for in some experiments a clean gap was eaten through a line, through which later arrivals could pass unscathed. With a massive band, it would be worth while to work on it continuously, laying short lines where hoppers seem to be leaking out, and ensuring that every hopper gets a chance to feed. Full-fed hoppers go to roost, and continued marching indicates the need for more bait.

Area baiting was done at estimated dosages of 8–16 gm./sq. metre (70–140 lb./acre). Wheat bran and the fine and medium grades of dura gave nearly 100 per cent. mortality within 24 hours. The mortality from the coarse fraction or kind of dura may have been a shade less or slower, but it was certainly very good.

Larger bands were also successfully line-baited with arsenic-maize bran, so it is clear that even those baits that were or seemed less acceptable in the tray tests were nevertheless good for control.

Summary.

Tests of the acceptability of crushed dura (*Sorghum* millet) of various particle size composition suggested only small differences between any of them and wheaten bran. There seemed to be a slight advantage to dura passing a sieve of 20 meshes to the inch and stopped by 30, and a slight disadvantage to dura stopped by a 20-mesh sieve. There is certainly no reason to use wheaten bran in preference to crushed dura.

Maize bran containing 2·5 per cent. sodium arsenite was less acceptable than the other materials, but crushed maize was not. Nevertheless, this arsenic bait and also coarse dura with BHC produced a mortality apparently no lower than the most acceptable baits containing 0·5 per cent. BHC.

It is suggested that experiments with milling and separating machinery may produce a first-class dura bait and at the same time reserve the flour for human consumption.

Acknowledgements.

I am grateful to Mr. K. Wilson Jones, Research Division, Wad Medani, for identification of plant material, to Dr. A. J. Henry, Government Analyst, for analysis of the dura baits, to Beshir effendi Babiker el Shafie and to Hassan effendi Idris whose excellent field organisation made it possible to conclude the work in the limited time available. In particular, I am grateful to Dr. D. L. Gunn who initiated the work on dry bait, and without whose help this paper would not have been written.

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THE GALL MIDGES OF ST. JOHN'S WORT (*HYPERICUM* SPP.), WITH DESCRIPTIONS OF TWO NEW SPECIES.

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Common St. John's Wort (*Hypericum perforatum* L.) is a noxious weed in parts of Australia and the U.S.A. In Europe, certain gall midges live on the leaves, others live in underground galls, while another group inhabits the flowers. In the U.S.A., one species attacks the flower buds and two others cause stem galls.

The purpose of this paper is to make readily available to those interested in the biological control of St. John's Wort a brief summary of the characters which serve to differentiate the gall midges of this weed. Incidentally, the flower-inhabiting species were not known until they were discovered by the late Stanley Garthside while he was investigating the insects of St. John's Wort in Europe on behalf of the Australian Council for Scientific and Industrial Research. Abbreviated descriptions of two of these species are included in this paper.

A knowledge of the host plant and the type of gall formed serves as a useful clue to the identity of most phytophagous gall midges. The next essential step is to rear the midges, and when this has been done a knowledge of the generic characters of gall midges usually suffices to distinguish the various species present on a particular plant species. Even then it is not possible, except in a few cases, to state with any degree of certainty the host-plant range of the species. This is largely the result of the tendency to associate a species of gall midge with the host plant on which it was first discovered and the lack of biological investigations. This omission is being slowly rectified and evidence is accumulating that while some species are severely restricted in their host-plant range, others have a much wider range.

The gall midges of St. John's Wort are no exception. If the galls are known and the midges are reared, their specific identity can usually be established, but with the

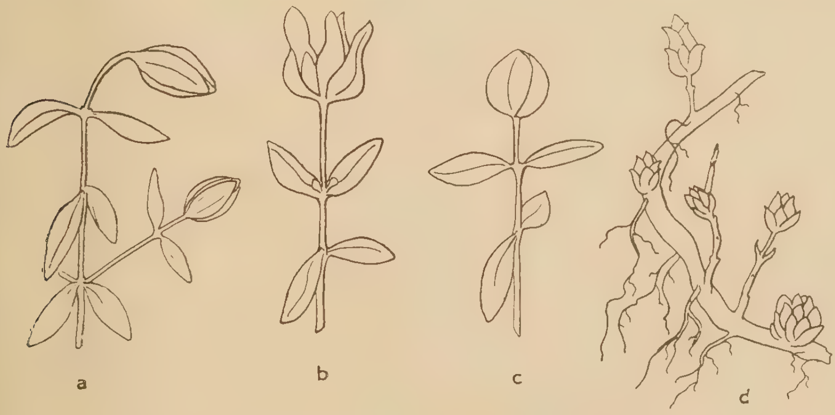

Fig. 1.—Galls of St. John's Wort gall midges: (*a*) *Dasyneura hyperici* (after Bremi); (*b*) *Dasyneura serotina* (after Houard); (*c*) *Zeuxidiplosis giardi* (after Rübsaamen & Hedicke); (*d*) *Geocrypta braueri* (after Handlirsch).

exception of one European species, little is known concerning their bionomics and although it seems likely that the species are at present restricted to *Hypericum*, it appears that no biological tests have yet been made.

A. Species living on the Leaves.

1. *Dasyneura hyperici* (Bremi, 1847).

The gall (fig. 1 *a*) of this species consists of a mass of terminal leaves drawn up together. The inner ones remain very small, while the outer ones are not noticeably malformed, nor are they discoloured to any extent.

The larvae are gregarious and are red in colour. Pupation takes place in the gall.

The adults are typical of the genus *Dasyneura*, i.e., the palps have four segments; the flagellar antennal segments are bead-like, with distinct necks in the males but not in the females (fig. 2 *a*); the third vein unites with the costa before the apex of the wing, the fifth vein is forked and the veins are not distinctly scaled; the claws on all the legs are toothed; the abdomen of the females is reddish with bands of black scales; and the ovipositor is pocket-shaped (fig. 4 *a*) and very extrusible. The male genitalia are typical of the genus, with well-defined upper lamella, lower lamella and harpes or ventral appendages. In the case of *D. hyperici*, the antennae consist of 2+12 segments in both sexes according to the original description and in all the specimens examined.

D. hyperici has been recorded from several species of *Hypericum* including *H. perforatum*. It is distributed all over Europe, including the British Isles.

2. *Dasyneura serotina* (Winnertz, 1853).

The gall (fig. 1 *b*) consists of the two terminal leaves of a shoot which remain small and covered by the penultimate pair; the latter are swollen and keel-shaped at their base.

The larvae are gregarious and white. Pupation takes place in the soil.

The midges themselves are very similar in all details to those of the previous species. Winnertz, in pointing this out, stated that *D. serotina* is slightly larger and the antennae have 2+14–15 segments. Without the evidence of the gall and colour of the larvae, this species would be exceedingly difficult to separate from *D. hyperici* because it is known that there is a tendency among *Dasyneura* species to vary as regards size and number of antennal segments according to food supply.

D. serotina has been recorded from several species of *Hypericum*, including *H. perforatum*, and appears to be distributed all over Europe, including the British Isles.

3. *Macrolabis marteli* Kieffer, 1892.

The gall is a tight, downward leaf roll. The rolled part is more hairy than the rest of the leaf.

The larvae are gregarious and white. Pupation takes place in the soil.

In general appearance, this species is rather similar to the two preceding species, but the adults have 2+10 antennal segments in the male and 2+11 in the female, while the flagellar segments are sessile in the male as in the female (fig. 2 *b*), whereas in the genus *Dasyneura* the male flagellar segments have a distinct neck. The most readily visible distinguishing features of the males are the sessile flagellar antennal segments and the remarkably swollen basal clasp segment of the genitalia (fig. 3 *b*). The females are difficult to distinguish from the *Dasyneura* species; when they are alive, however, their abdomens are orange yellow, whereas those of the two *Dasyneura* species are red. In both *Dasyneura* and *Macrolabis*, the claws are bifid on all the legs and the ovipositor is pocket-shaped.

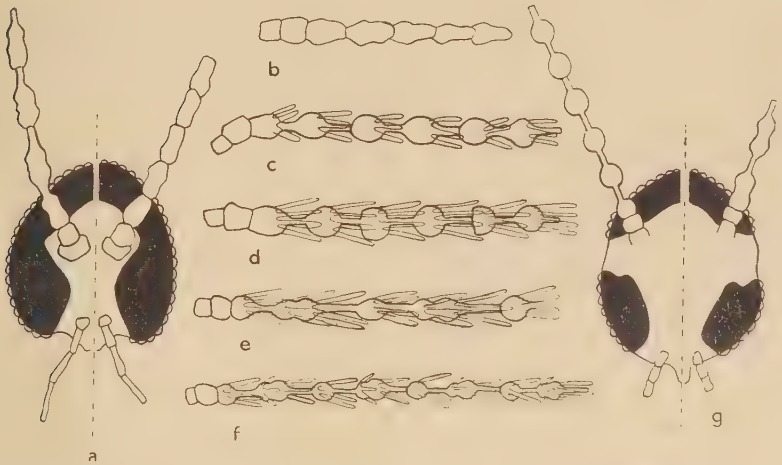

Fig. 2.—Diagrammatic representations to illustrate generic differences in the compound eyes and antennal segments of St. John's Wort gall midges ; (*a*) *Dasyneura hyperici,* male (left), female (right) ; (*b*) a *Macrolabis* species (*M. corrugans* F. Loew), male ; (*c*) *Zeuxidiplosis giardi,* male ; (*d*) *Contarinia hyperici,* male ; (*e*) *Clinodiplosis hyperici,* male ; (*f*) *Lestodiplosis* sp. from *Hypericum* flowers, male ; (*g*) a *Trisopsis* species (*T. tyroglyphi* Barnes), male (left), female (right).

M. marteli has so far only been recorded from France and England and only on *H. perforatum.*

4. *Zeuxidiplosis giardi* (Kieffer, 1896).

The galls (fig. 1 *c*) consist of incurved terminal leaves of shoots ; they are spherical and resemble fruits, and are usually red in colour.

The larvae are gregarious and red or yellowish-red in colour. They pupate in the galls. This is the only species of gall midge associated with *Hypericum* whose bionomics have been studied in any detail, and attempts have been made to ship it to Australia (Wilson, 1943).

Although the female midge has a red abdomen with dark transverse bands, thus bearing a superficial resemblance to the *Dasyneura* and *Macrolabis* species, *Z. giardi* is structurally quite different, belonging to the bifila group of the Cecidomyiariae and not to the Dasyneurariae. For example, the twelve flagellar antennal segments of the male are binodose with a circumfilum forming long loops on each node (fig. 2 *c*). In the female, the twelve flagellar segments are cylindrical with short necks, the proximal segment being obviously longer than the following one. The palps have three segments in both sexes. The claws on all the legs are simple without a tooth. The ovipositor (fig. 4 *c*) bears two large lamellae instead of being pocket-shaped as in both *Dasyneura* and *Macrolabis*. *Z. giardi* is also larger than the preceding three species.

Z. giardi has been recorded from several species of *Hypericum* including *H. perforatum* in several European countries, *e.g.*, England, France, Germany, Italy and Portugal.

B. Species living in the Flowers.

1. *Dasyneura toweri* Felt, 1909.

The gall consists of the axillary flower buds ; these become swollen, ovate and greenish or reddish in colour.

The larvae are yellowish, and Felt in his description stated that the anchor process or breastbone was wanting. This is quite exceptional for *Dasyneura* species and it is possible that a mutilated specimen was described. Presumably the larvae are gregarious although this was not actually stated.

The adults are of the usual *Dasyneura* structure. They possess 2+17 antennal segments.

D. toweri has only been recorded on *H. mutilum* and only from Magnolia, Mass., U.S.A.

2. *Contarinia hyperici*, **sp. n.**

This species occurred in samples of gall midges reared during August 1936 and July 1937 and sent in for identification by the late S. Garthside. A short technical description of both sexes is now given.

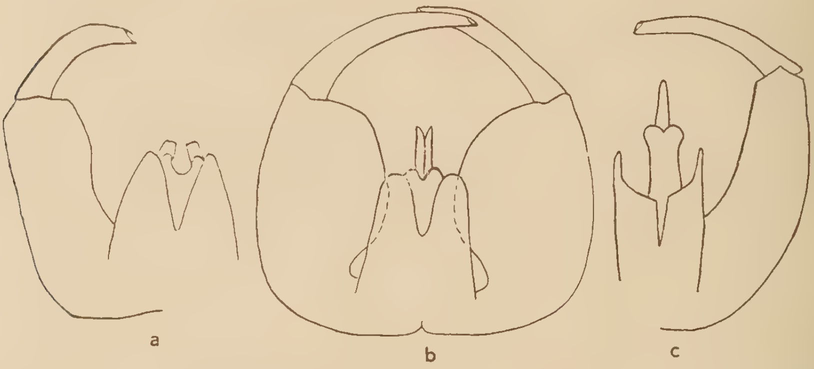

Fig. 3.—Diagrams illustrating the male genitalia of (*a*) *Dasyneura hyperici*; (*b*) a *Macrolabis* species (*M. corrugans*); (*c*) *Clinodiplosis hyperici*.

Male. Length about 1–1·25 mm. Antennae, 2+12, normal *Contarinia* structure, with distinct stems and necks of about equal width; stems of third flagellar segment about twice as long as broad, neck nearly three times as long as broad; loops of circumfila regular, overlapping the next node; stem of 10th flagellar segment about twice as long as broad, neck nearly three times as long as broad; stem of terminal flagellar segment about twice as long as broad, terminal node with pubescent protuberance distally, not much longer than broad. Palpi with four segments, proximal elongate quadrate, second about equal in length to third but slightly broader, distal segment about half again as long as third, proportions rather variable. Genitalia of typical *Contarinia* structure.

Cotypes; Cecid. 3417–20 inclusive, Cecid. 3419 with abnormal fusion of flagellar antennal segments (in the Barnes collection).

Other specimen; Cecid. 2829.

Female. Length about 1·25–1·5 mm. Antennae, 2+12, first flagellar segment about three times as long as broad, no neck, fused with second; second about two-thirds the length of first, about twice as long as broad, short transverse neck; third flagellar segment slightly shorter, about twice as long as broad, neck transverse, about twice as broad as long; succeeding segments slightly longer with necks increasing in length and decreasing in width; 10th flagellar segment slightly shorter, about twice as long as broad, neck about twice as long as broad; 12th longer than 10th, about two and a half times as long as broad, the usual distal prolongation scarcely separable. Ovipositor aciculate, very extensile. Otherwise about as in male.

Cotypes ; Cecid. 3421–8 inclusive (in the Barnes collection).

Other specimens ; Cecid. 2830–5 inclusive.

Habitat ; swollen flowers of *Hypericum perforatum* L.

Type locality : Box Hill, Surrey.

Other locality : Burnham Beeches, Buckinghamshire.

The gall of this species consists of flowers which remain closed and are slightly swollen. Instead of the flower bud being rather elongated and pointed at the tip just before it opens, those containing the larvae of this species are more squat, less pointed and usually rather swollen all around the base. The petals never open.

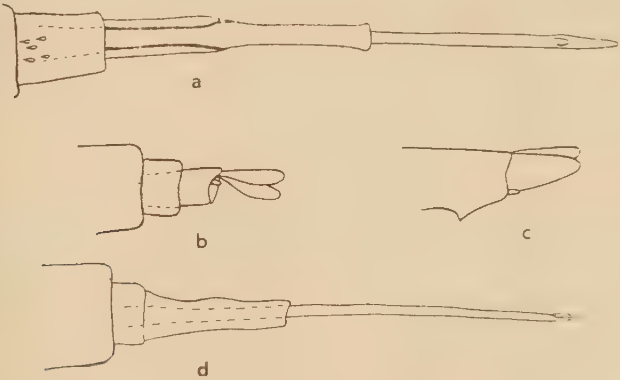

Fig. 4.—Diagrams illustrating the ovipositors of (*a*) *Dasyneura hyperici* ; (*b*) *Clinodiplosis hyperici* ; (*c*) *Zeuxidiplosis giardi* ; (*d*) *Contarinia hyperici*.

Pupation takes place in the soil. Garthside does not state the colour of the larvae in his letters, neither does he mention that the larvae " jump." It is probable that they do as this is characteristic of *Contarinia* species.

The midge belongs to the bifila group of the Cecidomyiariae and is of typical *Contarinia* structure. It therefore has four palp segments ; all the male flagellar antennal segments are binodose (fig. 2 *d*), each node bearing a circumfilum with long loops ; the claws are all simple ; the wings which are hyaline are neither especially broadened nor narrowed, the costa is not scaled, the third vein interrupts the margin at the apex of the wing ; the basal clasp segment of the male genitalia is not lobed and the ventral plate is deeply bilobed, while the ovipositor is needle-shaped, long and slender (fig. 4 *d*). The four palp segments and the shape of the ovipositor serve most readily to distinguish this species from *Zeuxidiplosis giardi*, which is also larger.

Contarinia hyperici has so far only been found for certain on *H. perforatum*, in Surrey and Buckinghamshire, England. Through the unfortunate loss of the correspondence* between Garthside and the writer concerning this species, Wilson did not know of this midge's existence and (1943) stated that the larvae of *Z. giardi* were occasionally found in small aborted flowers during the summer in the south of France. These larvae may have been those of *Contarinia hyperici*. Wilson also stated that " in England this insect (*Z. giardi*) normally develops in the flower-buds and does not produce leaf-bud galls, so two biological forms of this insect exist ". This last statement is an error because the flower-bud midges to which Wilson referred were in fact those now described as *Contarinia hyperici*.

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*Fortunately copies of the correspondence remained in the writer's possession.

3. *Clinodiplosis hyperici*, **sp. n.**

This species also was received from Garthside who reared specimens in September, 1929, from material collected at Burnham Beeches, Buckinghamshire, and again in September, 1936, from material collected at Box Hill, Surrey. In both cases the midges emerged from unopened flowers of *H. perforatum*. A short description of both sexes follows.

Male. Length about 1·25–1·5 mm. Antennae, 2+12, stem of third flagellar segment about three times as long as broad, neck about four times as long as broad; stem of 10th flagellar segment about four to five times as long as broad, neck five to six times as long as broad; stem of 12th flagellar segment about five times as long as broad, terminal protuberance slightly more than one-third the length of distal enlargement. Palpi with four segments, proximal segment small quadrate, second, third and distal long narrow, second about three times as long as broad, third and fourth each slightly longer than previous. Genitalia; typical *Clinodiplosis* structure.

Cotypes; Cecid. 2901–2 (in the Barnes collection).

Other specimens; Cecid. 1442–3 and 1446.

Female. Length about 1·5–2 mm. Antennae, 2+12, neck of third flagellar segment about one-third as long as basal enlargement and three to four times as long as broad; neck of 10th flagellar segment about half as long as basal enlargement and three to four times as long as broad; distal prolongation on 12th flagellar segment one-quarter to one-half the length of basal enlargement. Ovipositor lamelliform. Otherwise about as in male.

Cotypes; Cecid. 2903–6 inclusive (in the Barnes collection).

Other specimens; Cecid. 1444–5.

Habitat; swollen flowers of *Hypericum perforatum* L.

Type locality: Box Hill, Surrey.

Other locality: Burnham Beeches, Buckinghamshire.

This species is probably not a primary gall-maker, but lives in the galls caused by *Contarinia hyperici*.

The larvae were not distinguished by Garthside but are probably typical *Clinodiplosis* and as such could easily be separated by the anal segment from both *Contarinia hyperici* and the next species to be considered. Furthermore, *Clinodiplosis* spp. do not "jump", while those of *Lestodiplosis* are blood-red and have no anchor process.

The adults are readily recognisable since they belong to the trifila group of the Cecidomyiariae, *i.e.*, the nodes of the male flagellar antennal segments are plainly unequal and each flagellar segment bears three circumfila with long loops (fig. 2 *e*). It is a typical *Clinodiplosis*: the claws, which are bent almost at right angles, are toothed on the anterior and middle legs, while those on the posterior legs are simple; the palps have four segments; the circumfila are regular and have loops of approximately the same length; the basal clasp segment of the genitalia is not distinctly lobed, while the ventral plate is elongate and emarginate distally and the dorsal plate is deeply cleft (fig. 3 *c*). *Clinodiplosis hyperici* can easily be distinguished from *Contarinia hyperici* by the three circumfila on the male flagellar antennal segments, the elongate ventral plate or the lower lamella and by the lamelliform ovipositor (fig. 4 *b*).

Clinodiplosis hyperici has so far only been found on *Hypericum perforatum*, in Surrey and Buckinghamshire, England.

4. *Lestodiplosis* (*Coprodiplosis*) sp.

This is the third species reared by Garthside from unopened flowers of *H. perforatum*. The material was collected at Burnham Beeches, Buckinghamshire, during the summer of 1936. Although this species can be readily separated from the others living in unopened flowers of *H. perforatum*, it is considered wiser not to describe it as new since species in the genus *Lestodiplosis*, being predacious on other gall-midge larvae as well as on mites or acting as scavengers, are more likely to be polyphagous than are phytophagous species. One male (Cecid. 2822) and six females (Cecid. 2823–8) are in the Barnes collection.

Lestodiplosis sp. is, like *Clinodiplosis hyperici*, a member of the trifila group of the Cecidomyiariae, but may be easily distinguished by its smaller size, the shortness of the loops in the middle circumfilum (fig. 2 *f*), the short palp segments, the claws which are uniformly curved and all simple, the ventral plate of the male genitalia which is short, not elongate, and the basal clasp segment which has a distinct lobe. The ovipositor is lamelliform but the lamellae are smaller and differently shaped. The wings are hyaline and so this species would formerly have been placed in the genus *Coprodiplosis* and not in *Lestodiplosis* which was restricted to species having spotted wings. It is not known whether this species is predatory on either *Contarinia hyperici* or on *Clinodiplosis hyperici* or on both, or whether it is a scavenger.

5. *Another record of a flower-inhabiting gall midge.*

The only other known published record of a gall midge in the flowers of *Hypericum* is that by Bagnall & Harrison (1922) who stated that on *Hypericum pulchrum* in Northumberland and Durham they found gregarious glossy-white larvae with pinkish ends in flowers which remained closed, small and slightly thickened. In seemingly normal flowers they found single larvae. It is not possible to identify the gall midge from this information.

C. Species living in the Stems.

1. *Lasioptera virginica* Felt, 1909.

The gall of this species is described as being subglobose and monothalmous, with a length of 1 cm. and diameter of 8 cm. The large irregular inner chamber is surrounded by rather thick walls, the outer part of which is soft and the inner part irregularly hardened and blackened.

Only the female midge was described. It is a typical *Lasioptera* and can be separated from all other gall midges known to attack *Hypericum* by the characters which pertain to the Lasiopterariae, *i.e.*, the costa is thickly scaled, the third vein is very close to the anterior margin of the wing, and the antennal segments in both sexes are sessile, cylindrical and short. The characters which place it in the genus *Lasioptera* are : the third vein unites with the costa before the basal half, the palps have four segments, and the fifth vein is forked. In colour, these midges are conspicuous by reason of the silvery scales on the dark thorax and on the black abdomen and legs.

This stem gall has only so far been recorded on *H. virginicum* (Marsh St. John's Wort) and only at Magnolia, Mass., U.S.A.

2. *Cecidomyia* ? *triadenii* Beutenmüller, 1908.

This is a name given merely to a green, globular, somewhat elongate gall on the stalk of *Hypericum* (*Triadenum*) *virginicum* (Marsh St. John's Wort). The midge has not been described and has probably not even been reared. Felt (1925) stated that it was probably a synonym of *Lasioptera palustris* Felt which was originally described from an irregular stem gall occurring on a weed common in wet places at West Nyack, N.Y., U.S.A.

D. Species living in underground Galls.

1. *Geocrypta braueri* (Handlirsch, 1884).

The gall of this species is to be found on the underground shoots (fig. 1 *d*). It is oval or bud-shaped and consists of undeveloped leaves resembling scales. These latter are lanceolate, fleshy and tinged with yellow.

Two to five larvae live in each gall. They lie on the inner surface of some but not all of the swollen scales. Pupation takes place in white cocoons in the same position.

The adults emerge from mid-August to mid-September. *G. braueri* is superficially similar to species of *Dasyneura* and *Macrolabis*. In these three genera, the claws are bifid on all the legs and the ovipositor is pocket-shaped, but whereas the basal clasp segment of the male genitalia is swollen in *Macrolabis* (see fig. 3 *b*), in *Geocrypta* the terminal segment is swollen. According to Handlirsch, the antennal segments are 2+11 in the male and 2+12 in the female ; in the case of the male, the flagellar segments have necks and are not sessile, this providing a further character distinguishing this species from *Macrolabis* (see p. 698).

G. braueri has been recorded on *H. perforatum* and *H. pulchrum* in Austria, England and Italy.

2. *Geocrypta hypericina* Tavares, 1919.

The gall of this species appears to be exactly the same as that of the previous species, and the larvae, which according to Tavares are white, have identical habits as regards position within the gall and pupation.

The adults cannot be separated from *G. braueri* except by a specialist. Tavares distinguishes them by small differences in the number of antennal segments (2+12), in the shape of the fourth palp segment and chiefly because Handlirsch described the third vein in the wing of the male *G. braueri* as ending slightly before the point of the wing and in the female well before the point, whereas in *G. hypericina* the third vein ends at the same place in both sexes. Tavares stresses this latter difference. However, until the types or fresh specimens from the type localities have been examined it is difficult to be satisfied that *G. braueri* and *G. hypericina* are distinct species.

G. hypericina was described from specimens obtained on *H. pulchrum* at Redondela and near Pontevedra, Galicia, Spain.

3. *Trisopsis hyperici* Tavares, 1919.

This species was described from a solitary female midge reared from galls of *G. hypericina* collected at Redondela, Spain. Tavares, although mentioning that the male, pupa and larva were unknown, stated that this species lived as a commensal with the larvae of *G. hypericina*. Edwards caught specimens of what he thought was this species at Letchworth, Herts, England, on windows, but stated that it probably was commensal with other gall midges since there was no *Hypericum* in the near neighbourhood. If one may draw a parallel from the larvae of *T. tyroglyphi* Barnes, one may hazard a guess that those of *T. hyperici* are predacious.

T. hyperici may at once be distinguished from all other *Hypericum* gall midges by the fact that the compound eyes, although contiguous at the top of the head as usual, are not continuous laterally, the basal facets on each side being separated from the more dorsal ones. This gives the appearance of three eyes (fig. 2 *g*).

4. *Lestodiplosis* (*Coprodiplosis*) *hyperici* (Tavares, 1919).

The larvae of this species are red and live as predators in the galls of *Geocrypta hypericina*. They pupate in the soil. The characters given on p. 703 for recognising the *Lestodiplosis* (*Coprodiplosis*) sp. which lives in the unopened flowers of

H. perforatum are sufficient to distinguish *L. hyperici* from all other gall midges found on *Hypericum* except the *Lestodiplosis* sp. mentioned above. The fact that *L. hyperici* lives in the galls of *G. hypericina* will serve to distinguish it from the *Lestodiplosis* sp. reared from the unopened flowers. It is possible that only one species is involved, and future work may settle this point.

L. hyperici was described from galls found on *H. pulchrum* in Spain.

Summary.

Characters are given whereby the gall midges attacking St. John's Wort (*Hypericum*), a noxious weed, may be distinguished.

Four species, *Dasyneura hyperici* (Bremi), *D. serotina* (Winnertz), *Macrolabis marteli* Kieffer and *Zeuxidiplosis giardi* (Kieffer), inhabit leaf galls in Europe.

One species, *Dasyneura toweri* Felt, inhabits the flowers in the U.S.A. Three species, *Contarinia hyperici*, sp. n., *Clinodiplosis hyperici*, sp. n. and *Lestodiplosis* (*Coprodiplosis*) sp., live in the unopened flowers in Europe.

Two species, *Lasioptera virginica* Felt and *Cecidomyia ? triadenii* Beutenmüller, are recorded as attacking the stems in the U.S.A. The latter is a name given merely to a gall.

Four species, *Geocrypta braueri* (Handlirsch), *G. hypericina* (Tavares) (perhaps a synonym of *G. braueri*), *Trisopsis hyperici* Tavares and *Lestodiplosis* (*Coprodiplosis*) *hyperici* (Tavares), live in underground galls in Europe.

Descriptions of *Contarinia hyperici*, sp. n. and *Clinodiplosis hyperici*, sp. n. are given.

Acknowledgement.

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BIOLOGICAL OBSERVATIONS ON SOME THYNNIDS OF WESTERN PATAGONIA.

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(Plates XXIV & XXV.)

In the course of investigations of South American parasites of Melolonthinae conducted at the request of the Government of New Zealand in 1945–47, considerable attention was given to members of the Thynnidae, a Hymenopterous family generally regarded as being parasites of Scarabaeidae though the host preferences of only a few species are definitely known. The family is largely confined to Australia, but an important secondary centre occurs in the southern parts of South America, particularly Chile. For various reasons it was not possible to visit southern Chile, and observations were limited to the localities of San Carlos de Bariloche and El Bolson on the Argentine side of the southern Andes between latitudes 40 and 42°S.

The natural primitive plant associations of this area lying immediately east of the Andes consist of southern beech forest (*Nothofagus* spp.) and steppe of varying grass and shrub mixture. The former is considered an extension of the deciduous beech forest of southern Chile. In Argentina it is characterised by a discontinuous distribution and in the main occurs only in those parts where transverse valleys and passes permit the prevailing westerly rain-bearing winds to deposit a considerable part of their moisture within the Argentine frontier. The vegetation at the foot of the Andes is a consequence of the abrupt decrease in the rainfall rates, a grass steppe predominating nearer the Andes and in the lower sections of the mountain valleys, and shrub steppe which grades into Patagonian desert prevailing as one proceeds eastwards from the mountains into a region of rapidly diminishing rainfall. The similarity of the vegetation in the southern Argentine Andes and the adjacent piedmont area to that found on crossing the alps of the South Island of New Zealand has been remarked on by Davies (1940) in his account of the grasslands of Argentina.

In the steppe section, virtually all land is used for livestock ranching, particularly sheep, while relatively little of the natural forest cover has been cleared for agriculture in the mountain valleys. Consequently such agriculture as is practised there is in the main confined to areas of natural grassland and is barely sufficient to meet local needs.

The climate of the region resembles that of southern Chile between Temuco and Puerto Montt, but there are appreciable differences in rainfall and in winter temperatures. In Argentina the rainfall is less, particularly in summer, and the winter temperatures are lower.

It appears that no information has been published on the Thynnid fauna of the southern Andean area of Argentina other than a list and descriptions of seven new species in a publication on some Argentine Hymenoptera by Brèthes (1910). The papers of Durán (1941) and Janvier (1933) give useful information for the family for Chile. Durán's work is a systematic study of adults, while Janvier deals with biological observations on six of the more common species. Most of the species treated in the present paper also occur in Chile and it seems unlikely that the Thynnid fauna of western Patagonia differs greatly from that of southern Chile except in being poorer in species. It should be noted, however, that only a very small part of this territory was covered in the present investigation.

The following species were observed under field conditions and studied in as much detail as location of the habitat allowed.

Elaphroptera scoliaeformis (Hal.).
Pseudelaphroptera brevipilosa Durán.
Ornepetes semicincta Turner.
Telephoromyia (*Spilothynnus*) sp.
Elaphroptera (*Paralycus*) sp. near *nigripennis* (Smith).
Elaphroptera nigripennis (Smith).

The first four species occur typically in open areas such as natural pasture and cultivated land, whereas *E. nigripennis* and *E.* sp. near *nigripennis* are essentially forest inhabitants (Pl. XXIV). Of these latter species, *E.* sp. near *nigripennis* is common in fairly open *Libocedrus* woods, while *E. nigripennis* was only observed at higher altitudes near San Carlos de Bariloche in mixed forest where southern beech (*Nothofagus*) was abundant.

Telephoromyia sp. was found abundantly on pasture in the more arid steppe sections near Zapala, and *P. brevipilosa* has also been noted in a similar habitat in a more southern location, Los Repollos, situated between El Bolson and Maiten. The latter species, however, and *E. scoliaeformis* and *O. semicincta* were recorded in maximum numbers in open spaces in the forested valleys. Their distribution suggests that they were originally forest species which have recently spread to pasture.

Mating Flight and Feeding of Adults.

All the species listed above are characterised by apterous females which are carried on mating flights by the larger winged males (Pl. XXV, figs. 1 & 2). The time of occurrence of these spring and summer flights depends on the altitude of the habitat, the following table gives some indication of this variation.

Species	El Bolson (300 m.)	Repollos (500 m.)	San Carlos de Bariloche (870 m.)	Zapala (1,010 m.)
E. scoliaeformis	Oct., Nov.	—	Nov., Dec.	—
P. brevipilosa	Jan., Feb.	Feb., Mar.	Feb., Mar.	—
O. semicincta	Jan., Feb.	Feb., Mar.	Feb., Mar.	—
Telephoromyia sp.	—	—	—	Jan.
E. sp. near *nigripennis* ...	Nov., Dec.	—	Dec., Jan.	—
E. nigripennis	—	—	Nov., Dec.	—

Mating-flight behaviour is basically similar in all species. *E. scoliaeformis* may be taken to illustrate the general sequence of events. In the Bolson area, activity by this species over pasture is greatest on fine sunny days before noon, but some flights also take place in the afternoon and early evening. On one occasion pairs were easily secured on a showery, windy day. The males spend most of the day flying within six inches of the ground, retiring in late evening to near-by shrubs or trees for the night. They remain in such situations in cooler weather and in the afternoons of exceptionally hot days. The swarming of the males is generally confined to particular areas, which may be as much as several acres in extent, and they very quickly detect any female on the soil surface, particularly if it adopts the expectant pose which has been described by Janvier (*loc. cit.*) for several species of Chilean *Elaphroptera*. In this posture, the female climbs some plant stem, or stops at any point on the ground, and then elevates the prothorax and head by placing the forelegs close together beneath the thorax. The antennae, held forward and almost in contact, are lightly vibrated, while the mandibles are widely open. Several males quickly detect such a female and a struggle ensues until one male succeeds in carrying it off. The males approach the females against the wind and the attraction appears to be of an olfactory nature. Adoption of this calling posture does not invariably precede copulation; males sometimes locate females walking on the ground, exposed by a plough, or dropped by other males after the mating flight.

The successful male frequently has difficulty in renewing flight owing to the weight of the female, and usually climbs a small plant to gain elevation. After circling the spot several times at increasing height, the coupled male flies off to a section of the near-by *Libocedrus* forest, which in the Bolson valley is ¼ to ½ mile distant from the swarming grounds. Pairs have been observed to alight on trees bordering this forest but at heights too great to permit observation of behaviour. In one field in which there was a considerable infestation of this Thynnid, three sets of nine females each were marked by cutting off a part of the right metathoracic tarsus, and then released on consecutive days to be picked up by the males. Six of these females were later recovered in the same section of the field, the recoveries being two, one, and three, for the three successive days of the experiment. As it is difficult to keep track of all females being dropped in a given area on mornings of fair activity, the figures are probably significant and indicate a tendency for mated females to be returned to the field where they were picked up. As these marked females were recovered between one and two hours after release, the mating flight is of relatively short duration.

Pairs frequently pause on shrubs such as "calafate" (*Berberis* sp.) during the flights between open ground and forest, and on these, characteristic mating actions take place. The male gives a periodic, jerking, vertical motion to the abdomen, at the same time stroking the female with the hind legs. The female, when not being stroked by the male, has its head in continuous contact with the anal segments of the latter, these segments being grasped by the mandibles and palpated by the antennae. The female moves her head from side to side, and may at times grasp the male abdomen from above. Such actions perhaps serve to stimulate transfer of the spermatozoa.

The mating flight is terminated by the return of the male to open ground, where considerable circling takes place before the female disengages when the male momentarily touches the ground. The release is to some extent dependent on the female, as males have been seen to renew flight after alighting on the ground and trying unsuccessfully to disengage females by hind-leg movements. Females dropped by one male are sometimes again borne off on a second mating flight by another. On the other hand, females have been observed persistently to refuse males attracted by the calling posture. This behaviour is difficult to explain. It has been noted also with females of *E.* sp. near *nigripennis*.

In the case of *E.* sp. near *nigripennis*, the swarming males circle close to the forest floor throughout the day, but in this species the calling female does not maintain the mandibles in a fully extended position, and periodically partially closes them. This type of mandibular movement has not been noticed with any of the other Thynnid species investigated. The captured female is immediately borne aloft to the higher branches of nearby *Libocedrus* or *Lomatia* trees. Here it is usually difficult to follow the actions of such pairs, since they are located at least five yards from the ground. A few males have been observed to jerk and arch the abdomen after the manner of *E. scoliaeformis*, and mating activities of the two species are probably very similar.

It is of interest to contrast this action of a mating male in flying well above the forest floor with that of the unmated males which fly and rest within a few yards of the ground. The mating flight terminates with the descent of the pair to the forest floor where the male, after some circling, momentarily alights on the ground for the female to disengage. It seems likely that the male of this species, like that of *E. scoliaeformis*, returns the female to the locality where it was picked up. The flight of large numbers of males in the forest over areas where the females are about to emerge gives the impression of a sex ratio greatly in favour of the males, but this is not actually the case ; for example, one sample of 277 cocoons collected from the soil gave a subsequent emergence of 148 females and 124 males.

Swarming centres of *Pseudelaphroptera brevipilosa* and *Ornepetes semicincta* can only be located with difficulty in grassland owing to the small size of the males, but occasionally some dozens of males have been seen congregating over a small section of open pasture to pick up the females, and a few couples have been secured resting on blades of grass. The females ascend plant stems to a height of about twelve inches before adopting the characteristic waiting position. The males are very sensitive to the presence of a calling female, which is normally borne off within a few minutes after adopting this posture. This happened when females were released in a section of pasture where no males had been noted by the observer. Pairs are most easily secured from flower heads of the umbelliferous plants, *Foeniculum vulgare* and *Pastinaca sativa*, which are visited for feeding purposes. The females of both species are borne in the usual Thynnid manner, doubled up beneath the male abdomen, but the terminal segments of the latter are not grasped by the mandibles as in *Elaphroptera* spp. Only one female of *Pseudelaphroptera* was seen to be dropped over pasture after the mating flight. Nevertheless, it is clear that the females of the two species in question are carried from the flower heads to pasture or ploughed land and there deposited at the conclusion of the flight, but no conclusive evidence of a distinct migration to and from the feeding grounds of the type demonstrated for *Tiphia* spp. has been obtained.

No feeding by either sex of *E. scoliaeformis* has been observed on the shrubs or plants that flower abundantly in the spring. Some males have been caught with appreciable pollen on the body hairs, and an occasional specimen was noted in early summer on flower heads of wild parsnip (*Pastinaca sativa*). Possibly feeding takes place in the *Libocedrus* forest where pairs alight too high up for detailed observation. Examination of the alimentary tracts of pairs returning from the mating flight gave no positive evidence of feeding, since the contents of these differed little from those of pairs captured before starting the flight, but these latter pairs may have fed on previous days. The stomachs sometimes contained a clear fluid of honey-like consistency, and the rectum solid matter of an excretory nature, but the evidence for plant feeding by this species is inferential. Host feeding by females was not actually seen, but the collapsed condition of some hosts in the laboratory containers indicated that this had occurred.

The mouth parts of both male and female appear to be adapted for a suctorial or licking action (see Pl. XXV, fig. 3). The male mandibles are of a dentate type which might be of use in feeding, but their principal functions are cutting the cocoon wall for emergence and grasping the female for copulation. The female mandibles taper gently to a smooth, broad end* which cannot serve for piercing or biting but only for grasping, and they presumably serve mainly for grasping prey. The other mouth parts of both sexes are very similar and the components are shown in the figures. The mouth opening lies somewhat behind the base of the labrum, and immediately above it the epipharynx projects as a well-developed membranous flap. The maxillae and labium are united by membranes to form a composite structure which may be withdrawn so as to close the pre-oral cavity completely. In its extended position, this maxillo-labial complex forms a somewhat tubular structure, with the concave galeae of the maxillae overlying the bases of the glossae and paraglossae. Both the latter parts are cupped and appear to serve for the sucking or lapping of fluids, while they may also serve some purpose in the actions of the females during copulation described above.

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*In a ventral view of the head, the mandible appears as shown in Pl. xxv, fig. 3, but in a different view, it is seen to taper to a bluntly rounded extremity.

It is not known if pairs of *E.* sp. near *nigripennis* feed on exudations of *Libocedrus* or other plants during flights such as those observed in El Bolson. Janvier records such feeding for *E. nigripennis* on *Nothofagus obliqua* in Chile, but the writer's observations in the field and laboratory suggest that plants and host feeding are not extensive. The mouth parts of both sexes are as described for *E. scoliaeformis*.

Both sexes of *P. brevipilosa* and *O. semicincta* feed actively on flower heads of *Pastinaca sativa* and *Foeniculum vulgare* throughout the day from about two hours after sunrise to sunset. The male of each species moves rapidly over the flower head applying the mouth parts to the individual pistils. It periodically pauses at a pistil and jerks the abdomen for the female to move forward between its legs and feed. Feeding by the female is, however, very brief ; it almost immediately retreats and resumes its doubled-up position beneath the male abdomen. It remains in this position during most of the time spent by the pair on the flower heads. The male usually recommences feeding at the pistil fed on by the female before moving on to others. No evidence of host feeding was obtained. The mouth parts are of the same general pattern as that illustrated for *E. scoliaeformis*.

Host Relations.

Definite records of development on hosts under field conditions were obtained for only three Thynnid species. These are solitary external parasites, and their hosts are as follows.

Elaphroptera scoliaeformis	*Aulacopalpus pilicollis* (Fairm.) (Rutelinae)
Elaphroptera sp. near *nigripennis*	*Macrosoma glacialis* (F.) (Melolonthinae)
Pseudelaphroptera brevipilosa	*Adioristus acuminatus* Hust. (Curculionidae)

The Ruteline, *A. pilicollis*, is the common whitegrub in natural pasture and cultivated land of the region, and has a two-year cycle characterised by an early spring flight and late autumn or winter pupation. More sandy soils near lakes and rivers may also be infested by the Ruteline, *Hylamorpha elegans* (Burm.) but this species is pupating at the time of major flight activity of *E. scoliaeformis*. The third-instar larva of *A. pilicollis* is the normal, and apparently exclusive, host of this Thynnid.

E. sp. near *nigripennis* occurs in fairly open *Libocedrus* forest where the common soil-inhabiting whitegrubs are the Melolonthines, *Macrosoma glacialis* (F.), *Sericoides opacipennis* (Germain), *S. viridis* (Sol.), *S. delicatula* (Germain), *S. lineolata* (Germain), and *S. subcostata* (Germain). The mature larvae of *M. glacialis* are parasitised by *E.* sp. near *nigripennis*, which apparently does not attack the other species. This may be due in part to the small size of the mature larvae of these species, their average length being about half that of the full-grown *M. glacialis* larvae, which are 20 mm. long. The females of this Thynnid, when kept in small salve tins in the laboratory, often paralysed whitegrub larvae regardless of species or instar, but whereas eggs were readily laid on mature *M. glacialis* grubs, none was deposited on other species. The life cycle of *M. glacialis* covers two years ; there is a spring flight of adult beetles, and pupation occurs in late summer and autumn. Broods overlap considerably, second- and third-instar larvae often being found near adults resting in the cells in the autumn, but parasitisation has only been noted on the more mature third-instar larvae present in late spring and early summer and very occasionally on recently formed pupae.

The only known host of *P. brevipilosa* is the soil-inhabiting mature larva of the weevil, *Adioristus acuminatus* Hust. Other Curculionid larvae and larvae of the whitegrub, *Aulacopalpus pilicollis*, comparable in size to larvae of *A. acuminatus* that serve as hosts are also present in the soil of pasture land at the time of major female activity, but no case of their utilisation as hosts has been observed. *Ornepetes semicincta* is believed to attack the larvae of one of these Curculionid species, but this was not clearly established.

The foregoing data indicate a much more restricted host range than that given by Janvier for several other Thynnid species in his Chilean investigations. This author records species such as *Elaphroptera dimidiata* Guér. as parasitising larvae of as many as three Scarabaeid genera. He also observed that, for the purpose of feeding, *Elaphroptera* females readily attacked whitegrub larvae many times their size and produced injuries which later proved fatal. This predatory habit does not appear to be well developed in *E. scoliaeformis* or *E.* sp. near *nigripennis*, while the *P. brevipilosa* female is completely ineffective against any whitegrub larva of a size comparable to the normal host and is soon killed if confined in the usual laboratory containers with one of them.

Oviposition.

The females of *E. scoliaeformis*, *E.* sp. near *nigripennis*, *P. brevipilosa* and *O. semicincta*, on release from the male after the mating flight, lie quiescent on the soil surface in the doubled-up posture for about thirty seconds before attempting to enter the soil. Prior to egg-laying, they sting and permanently paralyse the hosts and malaxate them, but often some disordered movements of the mandibles, tail and legs may be observed. Stinging is sometimes so severe as to cause death. The method by which the female *E. scoliaeformis* attacks its host has been watched in gelatin capsules. Females of *E.* sp. near *nigripennis* and *P. brevipilosa* are very restless under these conditions and do not even paralyse their hosts in such containers; the sequence of actions jn these species probably follows that of *E. scoliaeformis*. The female of the latter, confined in a capsule with a mature *A. pilicollis* larva, requires a few minutes to settle down, passing to and fro over the whitegrub, but it eventually approaches the head of the larva and unhesitatingly grasps it in the region of the mouth with its mandibles, generally holding on to one of the host mandibles. In this attack, the whitegrub is apparently unable to injure the heavily chitinised Thynnid with its mandibles, and the female slowly curves its abdomen to insert the ovipositor in the ventral thoracic region of the whitegrub, between the legs. This grasping of the host near the mouth automatically ensures insertion of the ovipositor in the ventral thoracic region by a simple forward flexing of the abdomen of the Thynnid. The effect of the sting is very rapid ; gross movement of the grub finishes about 30 seconds later and the female retires from it to rest and clean herself. Several hours afterwards, the Thynnid begins malaxation of the whitegrub, and within 24 hours the abdomen of the latter is in a flaccid, fully extended condition instead of the normal, curved, turgid condition. In this preparation of the host, the Thynnid uses the mandibles and the first two pairs of legs, the metathoracic legs being placed for anchorage against the sides of the gelatin capsule and thus at right angles to the longitudinal axis of the host. This sequence of events is probably that followed in the soil in the feeding cell of the host. In laboratory experiments, some females attempted to bury the grubs and this habit may well account for the scarcity of cocoons of this parasite in the upper soil in fields where the flight of adults indicated some abundance.

The actual act of egg deposition has not been observed, but from the position of the egg on the mid-ventral abdominal line it is inferred that the female *Elaphroptera* straddles the host larva in the manner related above for malaxation. In the laboratory, eggs were laid 24–30 hours after the paralysing of the host and were placed longitudinally on the mid-ventral line of abdominal segments five to eight. Most eggs are found on segments six and seven, with the anterior pole facing forward. They are lightly attached to the hosts by the mucilaginous coating of the chorion. The egg of *E. scoliaeformis* is large, averaging 3 mm. long and 1 mm. wide. The

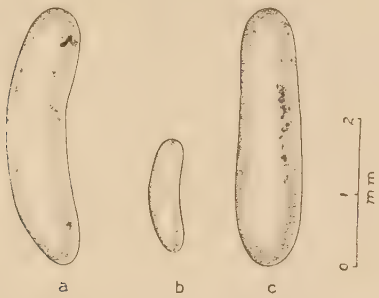

Fig. 1.—Egg of *Elaphroptera scoliaeformis* (Hal.). (*a*) lateral view ; (*c*) dorsal view ; (*b*) egg of *Pseudelaphroptera brevipilosa* Durán.

colour is a uniform white and the chorion thin and faintly reticulate. The general shape is indicated in fig. 1, and it will be noted that there is no pedicel for attachment to the host as described for *E. herbsti* André by Janvier.

E. sp. near *nigripennis* deposits the egg near the mid-ventral line of abdominal segments two to seven of the larva of *M. glacialis* ; it lies parallel with the host-body axis. Most eggs are on segments four, five and six. The egg is quite large, averaging 2·5 mm. long and 0·7 mm. wide ; in form, it is very similar to that of the preceding species. The broader cephalic end is generally directed towards the head of the host. The chorion is faintly reticulate and covered with a mucilaginous material which seems to be the only means of attachment to the host. Thus, as with *E. scoliaeformis*, dislodgement is very easy and it is exceptional to obtain field-collected hosts bearing eggs or young larvae.

The egg of *P. brevipilosa* is lightly cemented to the host by its caudal end and stands away from the host surface at an angle of about 45 degrees. Attachment in most cases is near the spiracular line of the *A. acuminatus* larva, between the third thoracic and sixth abdominal segments, but eggs are occasionally found on mid-dorsal and mid-ventral sections of the abdomen. The egg is white and measures 1·0–1·5 mm. long and the width is about a third of the length. The chorion is without sculpturing and coated with a mucilaginous material which often bears soil particles. Hosts bearing eggs are rarely found in the course of soil excavation because of the insecure attachment.

All the foregoing species deposit a single egg per host and no instance of super-parasitism has ever been noted. Laboratory experiments to determine the number of eggs laid by these species were largely unsuccessful. Females confined in large or small containers did not lay more than three or four eggs before they died some weeks later. Dissection showed some evidence of reabsorption in the ovarioles, but in many cases females that did not oviposit at all were found on later examination to contain one or two eggs apparently ready for deposition. The periodic mating flights in the field may serve to stimulate oviposition, but there seems little doubt that the species, even under the most favourable conditions, have low reproductive capacities.

Larval Development.

Laboratory rearing of the various immature stages is facilitated by the maintenance of uniform temperatures, but the following data were obtained at the varying summer temperature prevailing in the area.

In the three species studied hatching occurs in 4 to 6 days and is accomplished by a dorsal slitting of the chorion near the anterior pole, the egg shell being sloughed back off the body. The young first-instar larva of *E. scoliaeformis* and *E.* sp. near *nigripennis* makes a feeding puncture on the host surface at what was the point of attachment of the anterior end of the egg ; in the case of *P. brevipilosa* the puncture may lie anterior, posterior, or lateral to the point of attachment of the egg depending on the orientation of the latter. Very exceptionally a first-instar larva moves forward one or two segments on the host, but as a rule there is no such movement in this or any of the four succeeding instars. This contrasts sharply with the report of Burrell (1935) of the movements over the hosts by the young larvae of certain Australian Thynnids.

The larvae of all three species are similar in general appearance, and have the same principal characters in each of the five instars. The number of moults is easily ascertained, as the skins, which are sloughed off by a dorsal slit, lie as a pad on the ventral surface of the mature larva. The head capsule is well defined and lightly sclerotised, and there are 13 body segments, the segmentation being more evident in the thoracic region. The body is widest near the mid-abdominal region. The integument shows no punctation other than a faint punctate line which runs mid-dorsally along the body. This line is not often visible, as the larva is covered with a film of liquid excreted from a transverse slit in the last abdominal segment by a periodic flexing of the tail. Clausen (1940) quotes Illingworth as having observed a similar liquid produced from the tip of the body of larvae of the Scoliid, *Campsomeris radula* (F.). In the present case, the habit may serve a cleaning purpose, as particles of earth adhering to the Thynnid larva are soon carried off to one side. The respiratory system consists of two lateral tracheal trunks with spiracles borne on the prothoracic and first eight abdominal segments. The lateral tracheae are united by transverse branches in the regions of the neck and tail.

Feeding in the first four instars is by suction, with the mandibles used only for occasional piercing or rasping of the host integument. Viewed laterally when feeding, the mouth parts of a larva are seen closely pressed to the host surface and exerting a constant sucking action. Although the mandibles are often in movement, their points generally fall short of the host surface. The labrum, maxillae and labium are prominent lobe-like structures ; the mandibles are illustrated in fig. 2. Each mandible consists of a broad conical base which flattens abruptly to

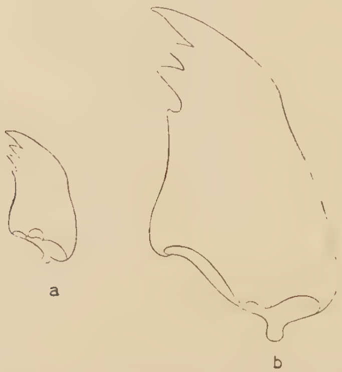

Fig. 2.—Mandibles of final-instar larvae of (*a*) *Pseudelaphroptera brevipilosa* Durán, (*b*) *Elaphroptera scoliaeformis* (Hal.) (×80).

form the blade-like cutting surface bearing the teeth. This structure of the mandibles is particularly evident in the first four instars, whereas in the fifth and final instar the form is a typical biting one. The mandibles are quadridentate in all instars of *E. scoliaeformis* and *E.* sp. near *nigripennis*. In *P. brevipilosa* they are tridentate in the first instar, but in the fifth instar they bear a small fourth tooth near the base of the third large tooth. Rudiments of this fourth tooth can be recognised in the earlier instars.

With each successive moult there is practically no change in the position of the feeding puncture of the instars. The fifth-instar larva tears a large hole in the host in the region of the earlier feeding punctures, and its head is embedded in the body cavity. Death of the host usually occurs a day or so before this. All of the grub except the head capsule is devoured by the two *Elaphroptera* species. *P. brevipilosa* often only partially devours its host before starting cocoon formation ; possibly this represents differential feeding of male and female larvae. There is little exudation from the feeding punctures comparable to that which has been described for various members of the SCOLIIDAE, and the liquid in which the Thynnid larvae are bathed is ejected from the tail of the larva in the manner related above. In a few instances it was noticed that a certain amount of liquid exudation was present over the terminal abdominal segments of first-instar larvae of *E. scoliaeformis* before any liquid was seen ventrally on the head and thoracic parts near the feeding puncture. Thus the feeding behaviour of these Thynnid larvae contrasts notably with that of Australian species observed by Burrell (1935) and rather resembles that of *Tiphia* species (Clausen, 1940).

Larval growth is rapid at the prevailing summer temperatures and terminates in 12 to 14 days. A short resting period of a few days intervenes before cocoon formation is commenced. The cocoons of the two sexes differ markedly, that of the male being long and thin-walled, and that of the female short and of more rigid construction. The difference in shape will be seen from the illustrations (fig. 3).

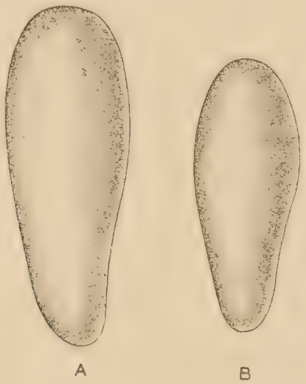

Fig. 3.—Cocoons of *Elaphroptera* sp. near *nigripennis* (Sm.). (*a*) male type ; (*b*) female type (×2·5).

The cocoon wall in each type can be fairly easily separated into three to six distinct layers, the number depending on the sex and species. The cocoons of *E. scoliaeformis* and *E.* sp. near *nigripennis* are three-layered in the male and six-layered in the female, while those of *P. brevipilosa* are three-layered in both sexes. The structures common to both types of cocoon are the outermost wall of dark, densely compacted, coarse silk threads, a layer of fine light-coloured silk, and an innermost layer of dark, somewhat coarser, silk. This last layer is absent from that portion of the anal extremity of the cocoon where the solid meconium is deposited. The greater rigidity of the cocoon of the female is due to a thickening of the outermost layer, but it also has the larger number of thin inner layers. The cocoon surfaces of the two *Elaphroptera* species bear externally a small amount of loose silk to which host remains are generally attached. These loose strands are part of the outermost wall. On the cocoon of *P. brevipilosa* they are so reduced that host remains are rarely attached.

The colour of the cocoons appears to vary according to soil conditions, but is usually a light brown. Cocoons with the extremity discoloured by the discharge of the meconium were always found to contain dead or dying larvae. The anal end of the cocoon normally contains the black, solid, meconial pad, and cast larval and pupal skins.

Emergence of the adult is effected by cutting an irregular hole near the anterior pole of the cocoon. A few cases have been observed of emergence from holes cut in the side near the pole.

Life-cycle.

Elaphroptera scoliaeformis, E. sp. near *nigripennis* and *P. brevipilosa* have a single generation each year, but there is evidence of persistence within the cocoon until the second year or even later. Incubation averages five days and larval feeding twelve days. The period from oviposition to cocoon formation generally covers three weeks. Then follows a period of at least nine months passed as a mature larva or pre-pupa within the cocoon. This diapause is not broken by changes of temperature. A certain percentage of overwintering cocoons of *E.* sp. near *nigripennis* contain fully formed adults of this species, and these can be induced to emerge normally simply by raising the temperature. This suggests that this species has a partial second generation in warmer climates. The adult life of the two *Elaphroptera* species after emergence from the cocoon probably covers two months, since field-collected females of unknown age lived under laboratory conditions for periods up to six weeks. Females of *P. brevipilosa* seem to be shorter lived than those of *Elaphroptera* spp. ; most field-collected specimens died in the laboratory within two weeks, though occasional specimens survived for a month.

The life-cycles of all species are closely synchronised with those of the hosts. This is particularly clear with *E.* sp. near *nigripennis* and its host, *Macrosoma glacialis.* The female Thynnids are active in the soil in the late spring and early summer, a time when the maximum number of mature whitegrub larvae are to be found prior to the autumn pupation.

Natural Enemies.

Extensive field collections have been made only with *E.* sp. near *nigripennis*, and this proved to be relatively free from attack by natural enemies. In samples of several hundred cocoons taken from the soil in different seasons, less than two per cent. were parasitised by a Bombyliid of the genus *Acrophthalmyda.* The larva of the Carabid beetle, *Cnemacanthus* sp., a predator of larvae of *Macrosoma* and other Scarabaeids, was occasionally observed in the field to attack a cocoon, but examination of the collections revealed that such attacks are very rare.

Swarming males are sometimes attacked by a predatory Asilid fly, *Lycomya germainii* Big., which mimics its prey. This fly has not been seen to attack mating pairs or unattached females. It is relatively uncommon in the areas investigated.

Discussion.

The Thynnid species treated in this paper, in common with other members of the family, present several points of particular interest. Two such points, namely, the function of the mating flight and the possible economic value of members of the family as controlling agents of Scarabaeid pests, may be briefly discussed.

The mating flight of the Thynnids has been regarded by various authors as serving for dissemination of the species and transportation to plant feeding grounds, in addition to the primary purpose of fertilisation. The data secured in the course of the present investigation suggest that the functioning of the flight for the first two purposes is questionable, at least in the case of the two species of *Elaphroptera*. They were never observed to visit flowers, nor was it possible to demonstrate feeding by them in the *Libocedrus* forest, although it is obvious that this environment has some definite attraction for the coupled males. Janvier reported that *E. dimidiata* and *E. nigripennis* fed in Chile on exudations produced in galleries of wood-boring insects attacking species of southern beech, but this has not been confirmed in the present survey. In regard to dissemination of the species, some evidence has been given to show that, after the mating flight, females may be returned to the sites where they were picked up. These Thynnid species, as well as their Scarabaeid hosts, are characterised by a very sporadic distribution in the field, and it might be suggested that the periodic mating flights serve effectively for the establishment of the species in new centres of host infestation. Observation does not support this view. It is not unusual to see mating pairs of *E. scoliaeformis* and *E.* sp. near *nigripennis* in flight far from the foci of congregating males, but it is perhaps significant that no females have been seen dropped in such locations. On the other hand, the release of females by males may be readily observed in the swarming centres, and this suggests that disengagement of the females does not normally occur except in the presence of other flying males. Undoubtedly some females are released in areas away from the swarming centres, but it is probable that such localities would be of low host incidence unless the males possess unsuspected powers of detecting heavy host infestations. It has been noticed that in the environs of El Bolson the principal foci of the two *Elaphroptera* species have occurred in the same sections of pasture and forest for several seasons, giving the impression that the Thynnid groups in such sites were maintaining themselves as more or less independent units. Thus, in view of the possible introduction of members of the family into other countries for biological control, the problem of the dissemination of the species by the mating flight would appear to merit further investigation.

As might be expected from the concentration of activity by the Thynnids investigated into relatively small circumscribed areas, parasitism by them may reach high figures. Burrell, commenting on the Thynnids as a group of economic value in the Australian fauna, cited species with an incidence of cocoons of ten to the square foot in the soil of restricted areas. In the Patagonian species examined, the maximum concentration found was twelve cocoons of *E.* sp. near *nigripennis* to the square metre, and this means that practically no host larva can have escaped attack in these areas. However, a sample of more than 1,000 larvae of the Melolonthine host gave an average parasitism estimate of only 23 per cent. Smaller field samples of the hosts of *E. scoliaeformis* and *P. brevipilosa* also gave indications of a degree of parasitism of this order. These species therefore appear to be useful but *not* spectacularly successful parasites. It is interesting to note in this connection that the Ruteline, *Hylamorpha elegans* (Burm)., one of the two most common Scarabaeids of the region, was not observed to be attacked by any Thynnid species. The somewhat limited observations of the present investigation suggest that Thynnids, in general, are not of great value as controlling factors of the local Scarabaeid fauna. It is known that several Scarabaeid species are of pest status in parts of southern Chile, and since Chile is one of the two world centres of Thynnids the area would appear to be more suitable for large-scale investigations to determine the value of the family as a controlling agent of whitegrubs. Dr. L. Durán of Santiago states, in correspondence, that the Ruteline, *Hylamorpha elegans* (Burm.), and the Melolonthines, *Sericoides germaini* D.T. and *Phytolaema herrmanni* Germ., are the injurious forms in natural pasture and cultivated land in the Cautin Province of southern Chile.

Summary.

During a survey for whitegrub parasites, six Thynnid species were found in parts of western Patagonia and studied in varying degrees of detail.

Definite host records are established for *Elaphroptera scoliaeformis* (Hal.), *E.* (*Paralycus*) sp. near *nigripennis* (Smith) and *Pseudelaphroptera brevipilosa* Durán, which are solitary external parasites of the mature larvae of *Aulacopalpus pilicollis* (Fairm.) (Rutelinae), *Macrosoma glacialis* (F.) (Melolonthinae) and *Adioristus acuminatus* Hust. (Curculionidae), respectively.

No adult feeding was observed during the mating flight of *E. scoliaeformis* and *E.* sp. near *nigripennis*, but pairs of *P. brevipilosa* and *Ornepetes semicincta* Turner fed extensively on flower heads of Umbelliferae. Some evidence is presented suggesting that at the conclusion of the mating flight the winged males tend to return the apterous females to the sites where they picked them up.

Hosts are permanently paralysed and malaxated before eggs are laid on them. The eggs of the two *Elaphroptera* species are placed parallel to the longitudinal axis of the host, on the mid-ventral line of abdominal segments two to seven. In the case of *P. brevipilosa*, the egg is lightly cemented by its caudal end at an angle of about 45 degrees to the host surface, the attachment being near the spiracular line of the host and between the third thoracic and sixth abdominal segments.

There are five larval instars. The larvae do not normally move over the host surface. In the first four instars feeding is by suction, but the fifth-instar larva tears a hole in the host in the region of the earlier feeding punctures. The film of liquid with which the larva is bathed is produced from its posterior extremity and is not exuded from the feeding puncture. Cocoons of two types may be recognised, corresponding to the sexes, the female type being the shorter and of more rigid construction.

E. scoliaeformis, *E.* sp. near *nigripennis*, and *P. brevipilosa* have a single generation a year. The period from egg deposition to cocoon formation is generally covered in three weeks, and at least nine months are spent in the cocoon as a mature larva or prepupa. Adults probably live up to two months.

The only natural enemies observed were a Bombyliid of the genus *Acrophthalmyda* and a Carabid predator, *Cnemacanthus* sp., attacking the cocoon stages of *E.* sp. near *nigripennis*. The adult of this Thynnid is also attacked by a mimicking Asilid fly, *Lycomya germainii* Big.

The function of the mating flight and the economic value of members of the THYNNIDAE as controlling agents for Scarabaeid pests are briefly discussed.

Acknowledgements.

The illustrations are by the artist, Sta. Sara Kahanoff of the Argentine Ministry of Agriculture, Buenos Aires.

The author is indebted to the following specialists for determination of the species dealt with. Thynnidae, Dr. L. Durán-Moya, Ministry of Agriculture, Santiago, Chile ; Diptera, Dr. E. E. Blanchard, Ministry of Agriculture, Buenos Aires, Argentina ; Coleoptera, Sr. Juan M. Bosq, Ministry of Agriculture, Buenos Aires, Argentina, and Sr. R. Gutierrez, Santiago, Chile.

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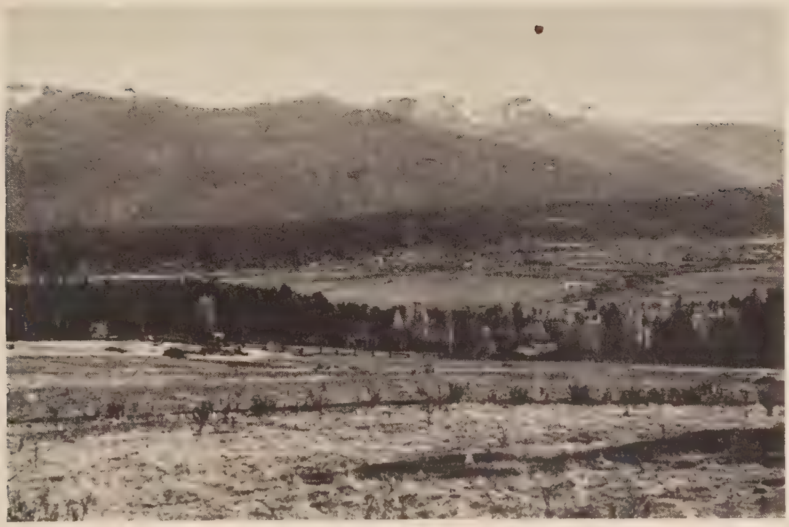


FIG. 1. Pasture habitat of *Elaphroptera scoliaeformis* (Hal.), *Pseudelaphroptera brevipilosa* Durán, and *Ornepetes semicincta* Turner.

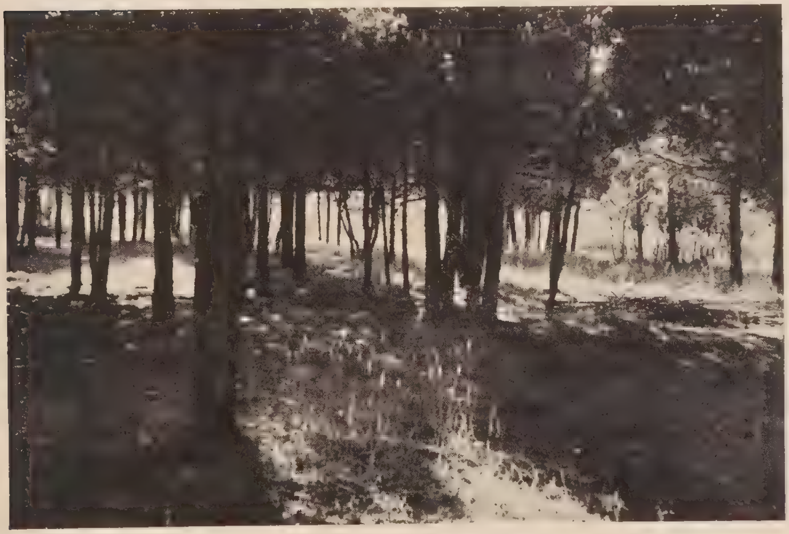


FIG. 2. Open forest habitat of *Elaphroptera* spp. near *nigripennis*.

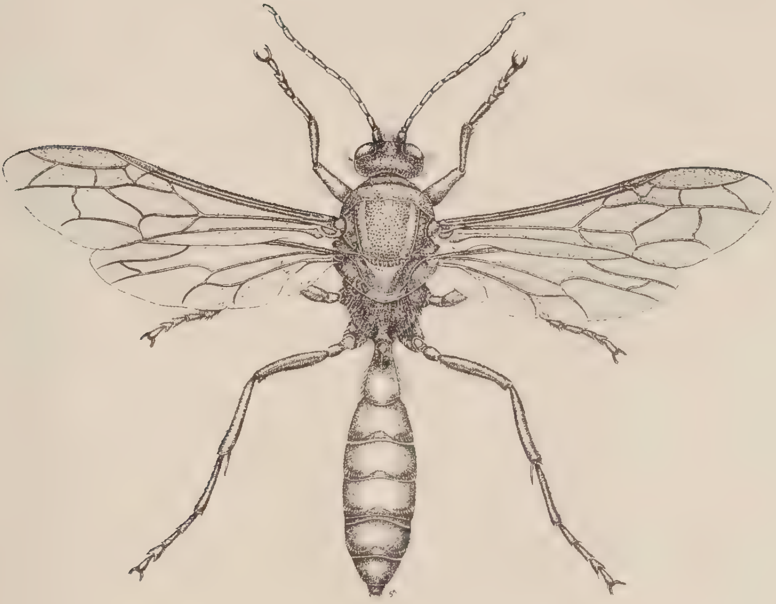
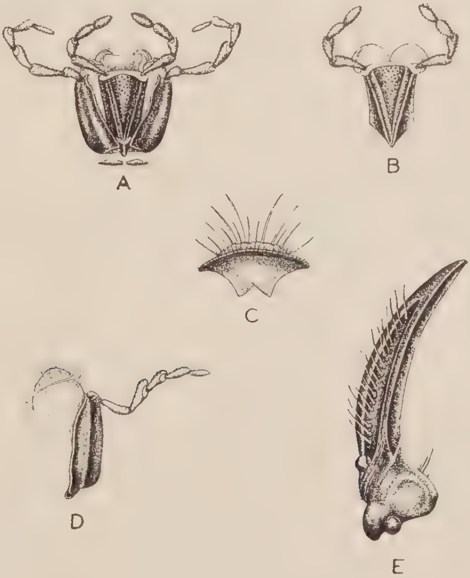


Fig. 1. *Elaphroptera scoliaeformis* (Hal.) ♂ (X 2.)



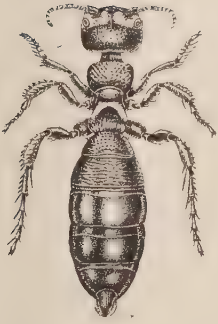


Fig. 2. *E. scoliaeformis* (Hal.) ♀ (X 2.)

Fig. 3. *E. scoliaeformis* (Hal.). Mouth parts. A. Maxillo-labial complex; B. Labium; C. Epipharynx; D. Maxilla; E. Mandible of female. (X 15.)

THE CREPUSCULAR BITING ACTIVITY OF INSECTS IN THE FOREST CANOPY IN BWAMBA, UGANDA.

A STUDY IN RELATION TO THE SYLVAN EPIDEMIOLOGY OF YELLOW FEVER.

By W. H. R. Lumsden.

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The length of the day in localities on or near the equator is practically constant. The same, however, does not apply to the times of sunrise and sunset which are subject to wide, though parallel, variation. Fig. 1, which is constructed from the data of the Nautical Almanac for 1949, shows these variations through the year as well as the similar variations in the times of Civil Twilight (Sun 6° below the horizon), Nautical Twilight (Sun 12° below the horizon) and Astronomical Twilight or darkness (Sun 18° below the horizon). Taking sunset as an example, it is latest (18.18 hours Local Mean Time) in the first part of February and earliest (17.47 hr. L.M.T.) at the end of October and beginning of November. The total variation is thus over a period of 31 minutes. Previous work in Bwamba on the cycles of biting activity of forest insects (Haddow, Gillett & Highton, 1947) has been related to the clock hours (L.M.T.) so that if a short peak of biting activity were closely related to sunset it might fall into one or other of two successive hours depending on the time of year at which the catch was performed. In making a close study of the crepuscular activity of biting insects it was therefore necessary to take these variations into account.

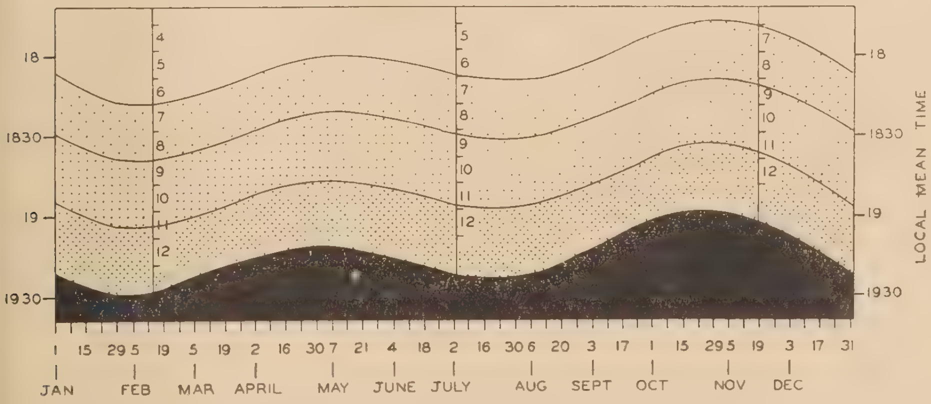

Fig. 1.—Curves showing the variations in the Local Mean Time of nightfall for an equatorial locality in 1949. The top curve is that of sunset and the lower curves in succession downwards are those of Civil, Nautical and Astronomical Twilight (Sun respectively 6, 12 and 18° below the horizon). Compiled from the data of the Nautical Almanac for 1949. The relations of some of the catch periods to these steps are also shown (Arabic numerals), for three representative dates, the 14th February, 3rd July and 18th November.

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Lumsden (1951a) reported on the sleeping habits of the monkeys occupying the same area as that in which the present work was carried out. The two studies may be taken together in assessing the exposure of wild monkeys to the attacks of various biting insects, a matter which is of importance in relation to the transmission of yellow fever virus among them. The studies may perhaps also be of importance in relation to other organisms parasitic in man, *e.g. Loa loa* (Cobbold) (Gordon & others, 1950).

Methods.

All the work was carried out at Mongiro (0°51′N., 30°08′E.), a small area on the edge of the Semliki Forest, Bwamba, Uganda, which has already been described in detail by Haddow, Gillett and Highton (1947) and Lumsden (1951a). Five tree platforms were available there, all being in swamp forest composed largely of *Mitragyna stipulosa* (DC.) O. Ktze. and *Elaeis guineënsis* Jacq. with only small numbers of *Pseudospondias microcarpa* (A. Rich.) Engl., *Macaranga schweinfurthii* Pax and *Ficus* spp. as the other main trees. *Mitragyna stipulosa* in this area is remarkably uniform in size and shape and composes, with the larger *E. guineënsis*, a dense canopy of foliage extending between about 15 and 30 metres above ground level. The forest floor is moist and is covered with a dense growth of Marantaceae and Zingiberaceae. All the platforms, numbered 1 to 5 from south to north, were in *M. stipulosa* except no. 5, which was in a *P. microcarpa*. They lay on a line, approximately 400 m. long, nearly parallel to a hillside ; four of them are marked on the sketch map of the area given by Lumsden (1951a). Their respective heights above ground level were 16·1, 17·6, 21·2, 18·2 and 18·4 m., *i.e.*, in the lower or middle canopy zone. Platform 1 was the least and platform 2 the most, shaded and sheltered by foliage.

The " bait " on each platform consisted of 3 native children, one of whom was appointed supervisor ; the whole five teams, one on each platform, were under a senior African supervisor and frequent visits were made to the work by the writer. The children caught all insects alighting on one another's skins in test-tubes, plugged the tubes with cotton wool and deposited them in bags numbered to correspond to the different collecting periods. When daylight failed collecting was continued by the light of a hurricane lamp. The timing of the catch was the charge of the senior supervisor who was supplied with an ordinary alarm clock for the purpose. These clocks were not very accurate but were set daily at the beginning of each catch by a clock sent down from the Field Station where an accurate chronometer was maintained, this being adjusted as necessary from wireless time signals, or, in default of these, by sunset observations. The catch timings were given by the supervisor to

Catch period	Time limits (" catch time ") (hr.)	Duration
1	15·00–16·00	1 hour
2	16·00–17·00	1 ,,
3	17·00–17·30	30 minutes
4	17·30–17·40	10 ,,
5	17·40–17·50	10 ,,
6	17·50–18·00	10 ,,
7	18·00–18·10	10 ,,
8	18·10–18·20	10 ,,
9	18·20–18·30	10 ,,
10	18·30–18·40	10 ,,
11	18·40–18·50	10 ,,
12	18·50–19·00	10 ,,
13	19·00–20·00	1 hour
14	20·00–21·00	1 ,,

the platforms by beat of drum. The catch clocks were not adjusted to Local Mean Time as this would have necessitated altering the timings of the catch as the L.M.T. of sunset altered through the year. They were adjusted to a special time, known as " catch time ", to which sunset always bore a constant relationship and which is used exclusively hereafter. The arrangement of each catch was as table, p. 722. In this arrangement sunset always coincided with 18.00 hr., *i.e.*, with the end of bag no. 6 and beginning of bag no. 7. The relation of some of the catch divisions to sunset and to the various standard twilight steps is shown by fig. 1.

The work was begun in February 1948, but at the beginning it was sometimes not possible to include all five platforms and some different timings were used before the arrangement described above was decided upon. These early catches, and occasional others in which all five platforms could not be included, have therefore been excluded from most of the present analysis which deals mainly with a total of 100 evenings on which catches were made on all five platforms by the method described. The 100 catch evenings were distributed as follows :—

1948	March	9–13, 16–20, 23–26, 30–31 ...	16 catch evenings
1948	April	1–3, 6–10, 13–15, 24, 27–30 ...	16 catch evenings
1948	May	1, 4–7	5 catch evenings
1948	November	2–6, 9–13, 16–20, 23–27 ...	20 catch evenings
1949	February	11–28	18 catch evenings
1949	March	1–25	25 catch evenings
		TOTAL ...	100 catch evenings

For some studies the catch evenings have been divided into five groups :—

Group 1	1948, 9 March to 3 April ...	19 catch evenings in 26 evenings
Group 2	1948, 6 April to 7 May	18 catch evenings in 32 evenings
Group 3	1948, 2 to 27 November... ...	20 catch evenings in 26 evenings
Group 4	1949, 11 February to 4 March ...	22 catch evenings in 22 evenings
Group 5	1949, 5 to 25 March	21 catch evenings in 21 evenings

It was not possible to make detailed meteorological observations during the catches, which were carried out for the main purpose of providing material for inoculation into susceptible animals in the search for yellow fever virus, but general notes of the climatic conditions, including cloud, wind and rain, were kept. The characteristics of the Bwamba climate as regards mean daily temperature and precipitation are shown by a climograph (fig. 2) based on observations made at the Field Station at Hakitengya which is 17 km. south of and 180 m. higher than Mongiro. Mongiro weather conditions may be expected to be in general slightly drier and hotter than those at the Field Station, but it should be noted that much of the Mongiro area is swamp which remains wet underfoot throughout the year.

The Species encountered.

We are indebted to Mr. H. Oldroyd of the British Museum (Natural History) and to Dr. F. van Emden of the Commonwealth Institute of Entomology, for the determinations of Tabanids and *Glossina*. Full names and the total numbers taken are given here for all the biting insects taken in the 100 complete catches and abbreviated names will be used subsequently. Unless otherwise stated all were females. The nomenclature used for the mosquitos follows Evans (1938) and Edwards (1941).

Anopheles (*Anopheles*) *coustani* var. *ziemanni* Grünberg	2
implexus (Theobald)	1
(*Myzomyia*) *funestus* Giles	2
demeilloni Evans	1
gambiae Giles	1,771
Aëdomyia africana Neveu-Lemaire	1
Taeniorhynchus (*Coquillettidia*) *metallicus* (Theobald)	1
maculipennis (Theobald)	6
fuscopennatus Theobald	191
aurites Theobald	4
microannulatus (Theobald)	1
(*Mansonioides*) *africanus* (Theobald)	428
uniformis (Theobald)	169
Aëdes (*Mucidus*) *nigerrimus* (Theobald)	126
grahami (Theobald)	10
(*Finlaya*) *longipalpis* (Grünberg)	9
ingrami Edwards	7
(*Stegomyia*) *simpsoni* (Theobald)	2
apicoargenteus (Theobald)	132
fraseri (Edwards)	1
africanus (Theobald)	841
luteocephalus (Newstead)	2
(*Aëdimorphus*) *tarsalis* (Newstead) group	3
abnormalis (Theobald) group	2
natronius Edwards	4
(*Diceromyia*) *furcifer* (Edwards)	8
Culex (*Culex*) *poicilipes* (Theobald)	1
Culex spp. indet.	203
Chrysops griseicollis Bequaert	1
centurionis Austen	1,946
Haematopota guineensis Bigot	2
Tabanus par Walker	1
socialis Walker	2
taeniola P. de Beauv. ♂	1
Glossina palpalis fuscipes Newstead ♂♂ 2 ♀♀ 5	7
TOTAL	5,889

Three males of the subgenus *Diceromyia* of *Aëdes* have now been taken in Bwamba and all have agreed with Edwards' (1941) description of the hypopygial characters of *furcifer* Edwards ; all records are therefore included under that species. *A.* (*D.*) *furcifer* was first recognised in Bwamba as a female on the 13th April 1948 and as a male on the 17th April 1948. It is considered likely that prior to these dates the species was included with *A.* (*A.*) *natronius* and therefore 13 records of earlier data have been omitted from Table XVIII, which therefore refers, as far as these species are concerned, to the last twelve days of catch group 2 and the whole of subsequent groups. Six other specimens which were damaged beyond the possibility of precise identification have also been omitted ; these were a large *Tabanus* sp. and five *Aëdes*, one of the subgenus *Mucidus*, the rest of the subgenus *Stegomyia*.

Methods of Analysis.

General statistical methods used are as described by Simpson and Roe (1939).

In the study of the differences between platforms and between evenings, the analysis of variance test was applied to tables showing the evening totals of each of

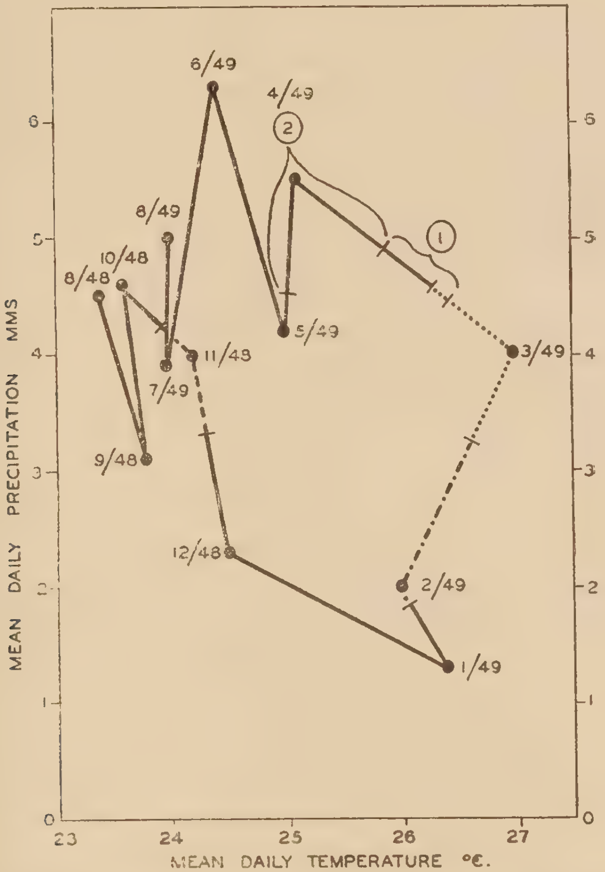

Fig. 2.—Climograph showing the mean daily temperature and precipitation at Hakitengya, Bwamba, for months from August 1948 to August 1949. The positions of the various catch groups are indicated ; groups 1 and 2 are shown by brackets and arabic numerals enclosing the corresponding dates in 1949 ; groups 3, 4 and 5 are shown on the actual climograph as dashes, dash-dots and dots, respectively.

the more numerous species on each platform. The numerical distributions of the catches of all these species were strongly positively skewed and hence logarithmic transformations might have been preferred. However, statistical advice on this point was to the effect that after such a transformation the degree of skewness is still large and that it is advisable, following Pitman (1938), to apply the analysis to the crude numbers.

Geometric means have been used where required in other studies, a treatment which follows the work of Williams (1937) who pointed out that the numerical changes which take place in catches of this sort are of a geometric nature, *i.e.*, a catch of one-quarter is as likely as one of four times the mean. It is necessary, as zero catches are frequent, to use the logarithms of (n+1) where n is the number of a given insect species taken on a single platform in the period under study. The geometric mean may be obtained readily from these figures as it is the antilogarithm of the arithmetic mean of the logarithms included in the calculation, minus unity.

The Abundant Species.

Differences between platforms.

Table I shows the total numbers of the eight abundant species taken on the five platforms over the whole series of 100 evening catches on each. The analysis of variance test shows highly significant ($P < \cdot 001$) differences between platforms for all species except *T.* (*C.*) *fuscopennatus* and *A.* (*M.*) *nigerrimus* ($P < \cdot 20 > \cdot 05$ and near ·20, respectively). In Table I the platforms are arranged in descending order of the total catches for each species. The differences between consecutive platforms in this order are often small and their significances have not been calculated ; it is considered sufficient to compare the first and last platforms and the general intermediate order. Platform 1 was the most, and platform 5 the least, favoured by *A.* (*M.*) *gambiae, T.* (*M.*) *africanus* and *T.* (*M.*) *uniformis* ; the orders of the intermediate platforms show only minor differences. The platform orders in the cases of *A.* (*S.*) *apicoargenteus* and *africanus* are closely similar to each other but differ from those of the three preceding species ; platform 2 yielded the most and platform 4 the least. Platform 1 yielded the largest catch of *Chrysops centurionis*, platform 4 the smallest.

TABLE I.

The total numbers of the eight more numerous species taken in 100 evenings between March 1948 and March 1949, on each of five tree platforms at Mongiro, Bwamba. For each species the platforms are arranged in order of total catch yielded.

Species	Total number of specimens caught in descending order. The number of the platform in each case is shown in brackets.					Total all platforms
A. (*M.*) *gambiae*	(1) 520	(3) 373	(4) 363	(2) 290	(5) 225	1,771
† *T.* (*C.*) *fuscopennatus*	(3) 57	(1) 43	(4) 39	(2) 28	(5) 24	191
(*M.*) *africanus*	(1) 135	(2) 101	(3) 94	(4) 65	(5) 33	428
uniformis	(1) 54	(2) 38	(3) 38	(4) 20	(5) 19	169
† *A.* (*M.*) *nigerrimus*	(3) 35	(5) 32	(4) 28	(1) 20	(2) 11	126
(*S.*) *apicoargenteus*	(2) 65	(5) 27	(1) 18	(3) 17	(4) 5	132
africanus	(2) 236	(3) 220	(1) 193	(5) 97	(4) 95	841
Chrysops centurionis	(1) 526	(3) 519	(2) 370	(5) 307	(4) 224	1,946
TOTAL	... ...	...	...	...	... ...	5,604

†Platform differences not significant.

The finding of such differences between simultaneous series of catches on five closely similar platforms, all in a small area of forest, is of interest. It is unlikely, in view of the various results for the several species, that these differences arise from catching teams of different degrees of efficiency. In any case, although no planned random distribution of the catching boys was made, shuffling was constantly taking place. The results may be compared with the obvious characteristics of the several platforms. Platform 1, that most productive of *A.* (*M.*) *gambiae*, *T.* (*M.*) *africanus* and *T.* (*M.*) *uniformis* was also the lowest platform and that least shaded and sheltered by foliage. Although all these three species tend to prefer the lower levels for biting (Haddow & others, 1947), height of platform alone is not an adequate explanation as platform 3, the highest one, consistently occupied second or third place. Further, as far as shade and shelter by foliage are concerned, platform 2, the most shaded, yielded a poor catch in the case of *A.* (*M.*) *gambiae* only. It may be noted that all these species are largely, or even entirely, derived from breeding places outside the forest area studied and whose breeding places are in, or associated with, the extensive areas or treeless swamp from which none of the platforms is distant more than 150 m. *A*. (*M*.) *gambiae* has been found as a larva in this area and *T*. (*M*.) *africanus* and *uniformis* are typically associated with open swamps (Hopkins, 1936). With regard to *A*. (*S*.) *apicoargenteus* and *africanus* there is again no apparent relationship between the obvious characteristics of the several platforms and the size of the catch. Both these species breed in tree-holes within the forest. Neither do the obvious characteristics of the platforms bear any apparent relationship to the size of the catch of *C*. *centurionis*. This species probably also breeds in forest ; the breeding places of the closely allied West African species are typically densely shaded (Gordon & others, 1950).

The order of the platforms as regards the numbers of any given species produced seems to be related to the breeding habits of the species in question rather than to the characteristics of the platforms themselves ; the orders found tend to be the same in species of similar breeding habits. From this it may be deduced that the catch on any given platform is determined mainly by the local population of the insect which in turn will be related to the local abundance and proximity of breeding places.

In the case of *C*. *centurionis* it is possible to test this hypothesis as, unlike the situation in the mosquito species, the production of adults from pupae appears to be confined to a short period yearly (see p. 731). If the hypothesis is true then it would be expected that during the period of hatching there might be marked differences between the platforms and that during the rest of the year the population would be much more evenly distributed. The numbers taken on each of the five platforms during the hatching period and during the rest of the catch series were :—

Platform	Hatching period (17 March–10 April 1948)	Rest of series	Totals
1	384	142	526
2	218	152	370
3	348	171	519
4	146	78	224
5	136	171	307

Applying the analysis of variance test to the original data shows that the variance due to platforms is significant in both cases but is more highly so in the hatching period ($P<\cdot001$) than in the rest of the series ($P<\cdot01>\cdot001$). The same test has also been applied to four platforms, platform 4 being omitted, and while the variance due to platforms during the hatching period was again significant ($P<\cdot001$), that during the rest of the series was not, the Between Platforms mean square being actually less than the Residual mean square. The hypothesis is therefore strongly supported, only one platform deviating largely from the result expected.

Seasonal variations in abundance.

The analysis of variance indicates that the differences between days are highly significant ($P<\cdot001$) for all the species. The variations in numbers of seven species through the whole series are shown in fig. 3. The values presented are three-day moving geometric mean catches per platform per evening. When the catch series was continuous, as in catch groups 4 and 5, the three days were, of course, successive. In the other catch groups the series was not continuous. The longest gap was between the 15th and 24th April, 1948 but in this interval catches were made on the 16th, 17th and 20th–23rd April and were identical with the others except that one or two platforms could not be included ; platform 2 was omitted on all these evenings and platform 3 also on the 20th to 23rd April. These evenings have been included here in order to bridge this otherwise rather large gap but values plotted from them in fig. 3 are distinguished as based on a smaller number of platforms than the remainder. The numbers of the insects at present under discussion in the incomplete catches were :—

A. (*M.*) *gambiae*	148
T. (*C.*) *fuscopennatus*	2
(*M.*) *africanus*	18
uniformis	5
A. (*M.*) *nigerrimus*	26
(*S.*) *africanus*	44
Chrysops centurionis	71
TOTAL	314

When these catches are taken into account the maximum number of successive evenings on which any three-day mean is based is six.

The use of the geometric mean of several days, covering a greater variety of weather conditions than a single day, will tend to smooth the effects of changes in activity and provide an index of the size of the population during the period included. With mosquito populations, which are subject to rapid changes, it has seemed advisable to base the extended mean on a short period and three days was selected. It should be borne in mind that a period of this length will not, in Bwamba, remove entirely the effects of weather as spells of similar weather, wet or dry spells, may sometimes last a week or more. Further, the catches sample only one level, the forest canopy, and do not cover the whole twenty-four hours of the day so that spurious population trends might be caused by changes in the time or level of activity of the insects under study. For instance, Haddow & others (1947) have produced evidence that a given species tends to retreat to its optimum habitat in unfavourable weather ; in studies of the biting mosquito population at several levels, including the ground, they took 46 per cent. of the total catch of *A.* (*S.*) *africanus* in the forest canopy in the wet season, 65 per cent. in the dry weather. They did not, however, in the same work find any evidence of marked changes in diurnal periodicity (Haddow—personal communication). In any case, the present work, lacking a concurrent, counter-attracting, bait of catchers at ground level, imitates as closely as possible the conditions to which resting monkeys are exposed and the results may be taken as representative of the seasonal variations in attack by the various species on monkeys in the forest canopy, which is one of the basic matters under investigation.

Fig. 3 also shows the rainfall at or near Hakitengya during the time of the catch series. The amount of rainfall before the parts of the catch series began, although not shown, is also of interest. In the 60 days preceding the 10th March 1948 the total rainfall was 169 mm. (of which 79 mm. fell on one day, the 7th March 1948) or 2·82 mm. per day. Similarly before the 3rd November 1948 the total rainfall for 60 days was 229 mm. or 3·81 mm. per day and before the 12th February 1949, 126 mm. or 2·1 mm. per day. As far as rainfall is concerned catch group 4 was preceded by a more rigorous dry period than was catch group 1 ; catch group 3 was preceded by a time of fairly abundant rainfall.

A. (*M.*) *gambiae.* The total numbers of this species taken in catch groups 1 to 5 were, respectively, 181, 514, 781, 224 and 71. It was least abundant in the latter part of March in both 1948 and 1949 an effect almost certainly to be related to the reduction of suitable breeding places by the dry weather. Its numbers increased rapidly in the first half of April, 1948, and reached a high level about the 20th April.

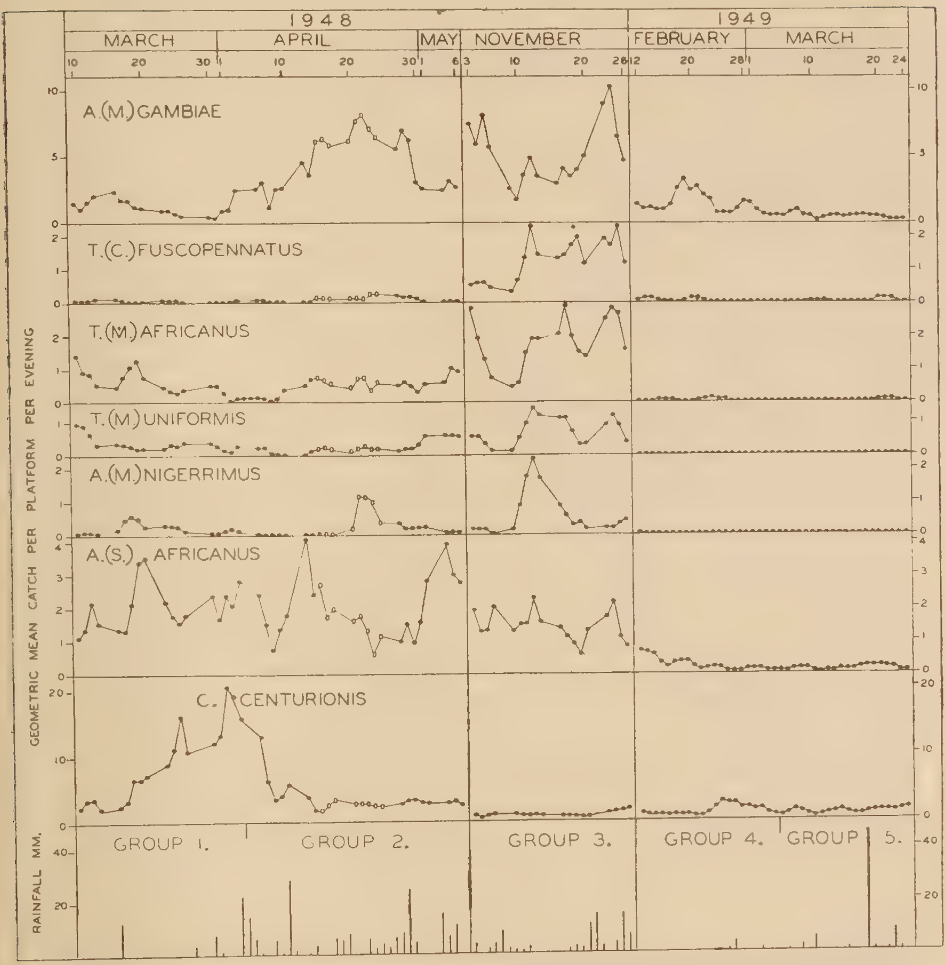

Fig. 3.—Seasonal changes in the numbers of seven of the more numerous species. The values plotted are three-day moving geometric mean catches per platform per evening and each is therefore usually based on 15 examples; eight values, based on smaller numbers of examples are distinguished as open rectangles. Rainfall during the catch series is also shown.

	Catch group				
	1 9 March– 3 April, 1948	2 6 April– 7 May, 1948	3 2–27 November, 1948	4 11 Feb.– 4 March, 1949	5 5–25 March, 1949
T. (C.) fuscopennatus... ...	4	7	173	4	3
(M.) africanus	89	63	272	3	1
uniformis	46	32	91	0	0

Thenceforward until the end of catch group 2 and again in catch group 3 the numbers reached high levels on several occasions but were subject to large fluctuations.

Taeniorhynchus spp. None of the three species of this genus was very abundant except towards the end of the wet season, in catch group 3, when all attained their highest levels. The total numbers of these species taken in the several catch groups are as shown on p. 729.

In the dry season of 1949, slightly more rigorous as regards drought than that of the previous year, all three species virtually disappeared from the catches. The short term variations in numbers in catch group 3 are roughly parallel in all three species and also show some correspondence to the changes in *Anopheles* (*M.*) *gambiae* and *Aëdes* (*M.*) *nigerrimus*. In all these species there is a more or less well-marked rise in numbers after the 10th November. The weather of November 1948 exhibited two successive wet spells separated by an interval of dry weather from the 12th to the 16th of the month. This is clearly shown by the rainfall distribution in fig. 3. The mean catches of these five species increase about the beginning of the dry interval which might simply be ascribed to more favourable biting conditions during that time. However, even taking into account the spreading effect consequent on the use of 3-day means, the rise is evident in 4 of the species before the end of the wet spell. Further, the subsequent courses of the curves do not correspond with such a hypothesis ; in four species the high level is generally maintained, or even increased, in the second wet spell. It is likely that the greater numbers after the 10th November are due to increases in the populations of these species, all at about the same time. It may be noted that their breeding places are probably associated ; the species of *Taeniorhynchus* breed in swamps (Hopkins, 1936 ; Gillett, 1946), and pools near swamp edges and in marshy ground are quoted as characteristic breeding places for *A.* (*M.*) *gambiae* (Evans, 1938) and *A.* (*M.*) *nigerrimus* (Hopkins, 1936), respectively.

A. (*M.*) *nigerrimus*. The total numbers of this species taken in catch groups 1 to 5 were, respectively, 25, 13, 87, 1 and 0. It was more markedly reduced in numbers in catch groups 4 and 5 than in catch group 1, probably because of the greater rigour of the 1949 dry season. The sudden increase in numbers in catch group 3 was a dramatic occurrence. The geometric mean catches per platform for the five successive evenings 9th–13th November were, respectively, 0, 0·43, 3·49, 2·28 and 1·67 ; the three latter nights accounted for 55 of the total of 126 specimens taken over the whole catch series. This rise in numbers has been discussed in conjunction with the *Taeniorhynchus* spp. above.

A. (*S.*) *apicoargenteus*. The variations in number of this species have not been shown in detail as it is active mainly in the daylight hours (Haddow & others, 1947) and its activity is decreasing even in the early catch periods (p. 740). The total numbers taken in catch groups 1 to 5 were, respectively, 37, 66, 19, 8 and 2. It was most abundant in the beginning of the wet season of 1948 (catch group 2), less so at its end (catch group 3) and was reduced to low levels in the dry season of 1949 (catch groups 4 and 5).

A. (*S.*) *africanus*. The total numbers of this species taken in catch groups 1 to 5 were, respectively, 282, 281, 226, 34 and 18. It was abundant throughout catch groups 1 and 2, though there were wide fluctuations, and was still common in catch group 3, towards the end of the wet season, but catches then were generally at a lower level. During the dry season of 1949 (catch groups 4 and 5) it was scarce, being reduced to much lower levels than it was in the corresponding period of 1948 though the drought was only slightly more rigorous. This reduction is considered to be due to a population decrease rather than to a depression of activity as no immediate increase was apparent at the onset of the wet season towards the end of March, 1949. Further, the appearance of fresh, undamaged, specimens of *A.* (*S.*) *africanus* was very noticeable ; the first was recorded on the 13th March 1949, and of the 13 specimens taken subsequently only 2, taken on the 15th and 16th March, were other than fresh and undamaged. The 16 days immediately preceding the 13th March

1949, may, therefore, be taken as representative of the minimum *A.* (*S.*) *africanus* population during the dry weather and compared with the findings towards the end of the previous wet season, in catch group 3. The geometric mean catch of *A.* (*S.*) *africanus* per platform per evening was 1·387 in catch group 3 and 0·059 in the 16 days prior to the 13th March 1949 ; the relation between these means is as 23·5 : 1. There is thus some evidence that although *A.* (*S.*) *africanus* may be found as an adult throughout the dry season in Bwamba (the maximum number of successive evenings on which the species did not appear on any individual platform was 34 but over all the platforms, only 3) the diminution which the adult population suffers is a severe one, at least in some years.

C. centurionis. The total numbers taken in catch groups 1 to 5 were, respectively, 1,077, 451, 118, 165 and 135. The species was present throughout the catch series but was least abundant in catch group 3 ; there was only one evening (in catch group 3) on which it failed to appear. Its period of greatest abundance was between the 17th March and 10th April 1948, with a maximum on the 1st April, on which evening a total of 194 was taken. Haddow (personal communication) has analysed the data presented in Haddow & others (1950) in another manner for comparison with the present results ; a total of 240 *C. centurionis*, taken on five platforms nearly identical to those used in the present work, in the months April to June 1945 was distributed as follows :—

Dates of catches	Number *C. centurionis* per 100 man-hours
10–14 April	80·0
17–21 April	20·0
23–28 April	18·3
30 April–5 May ...	8·3
7–12 May	2·5
14–19 May	1·8
21–26 May	2·0
28 May–2 June ...	1·2
4–9 June	1·5
11–16 June	0·0
18–23 June	2·3

It will be noted that in 1945 a marked decrease in numbers took place in April though at a slightly later date than in 1948. This effect almost certainly corresponds with the latter part of the curve defined wholly in 1948 and indicates that this outbreak is probably a yearly event. Although there are considerable parts of the yearly cycle still uncovered by Haddow's and the present catch series, the periods selected are representative and there is no sign of a similar increase in numbers at any other time. It may be taken as a tentative conclusion that the main period of pupal hatching for *C. centurionis* in Bwamba is in March and April. This conclusion will be supported by evidence to show that during the period of greatest abundance the population consists of two distinct groups, one of which tends to bite in the earlier catch periods, the other after sunset ; the latter group is that mainly in evidence at other times in the catch series. The occurrence of two population groups might then be explained by the presence at this time of a large proportion of newly-hatched individuals, insignificant at other times ; the habits of an insect at its first, and subsequent, blood meals may differ.

The beginning of the increase in numbers of *C. centurionis* antedates the onset of the main wet season by about nine days (fig. 3) but it may well be related to the isolated heavy rainstorms which occurred on the 7th and 16th March 1948 in

Bwamba. After reaching a maximum, the numbers of *C. centurionis* return to low levels in about a week which seems to be a more rapid reduction than could be accounted for by mortality alone ; probably the platforms at Mongiro are close to important breeding places whence the insects distribute themselves over large areas of forest. Streams of the type described by Gordon & others (1948, 1950) as characteristic breeding places of *Chrysops silacea* Austen and *C. dimidiata* Wulp in West Africa are more abundant at Mongiro than at any other of the many parts of the forest boundary in Bwamba familiar to the author.

The effect of weather on the magnitude of the catches.

The large, long term, fluctuations in the numbers of each species taken, into whose causation changes in population enter largely, complicate the study of this matter. However, large changes of population are less likely over short periods and differences in the size of the catches over a period of a few days are likely to be caused mainly by activity changes related to the meteorological conditions prevailing. The geometric mean catch of a given species per platform for an individual evening has therefore been compared with the same mean based on the three evenings centred on the evening in question. The incomplete catches of the 16th and 23rd April 1948 (p. 727) have been utilised here so that the maximum number of successive evenings on which any three-evening mean is based is again six. In this way the evenings were divided into two categories according as the mean catch of a given species for an individual evening exceeded, or fell short of, the mean based on the three evenings. This procedure was carried out for the three most numerous species only, *viz.*, *A.* (*M.*) *gambiae*, *A.* (*S.*) *africanus* and *C. centurionis* ; 94 or 92 comparisons are available ; six evenings were eliminated as the initial or terminal evenings of catch groups 1 and 2, 3, and 4 and 5 ; further, on two occasions in the case of *A.* (*S.*) *africanus* the evening's mean equalled the mean of the three evenings and these comparisons have been discarded.

TABLE II.

The distribution of geometric mean catches per platform tor individual evenings above and below the same mean based on the three days centred on the evening in question. Comparisons of three species by pairs.

		A. (*M.*) *gambiae*			*C. centurionis*		
		Above the mean	Below the mean	Totals	Above the mean	Below the mean	Totals
A. (*S.*) *africanus*	Above the mean	24	18	42	21	21	42
	Below the mean	16	34	50	24	26	50
	Totals	40	52	92	45	47	92
C. centurionis	Above the mean	22	25	47			
	Below the mean	20	27	47			
	Totals	42	52	94			

The species are first compared with each other as pairs in Table II to investigate whether evenings producing catches above the mean for one species of a pair tend to do likewise, or opposite, for the other. The values of χ^2 and the corresponding probabilities for these arrangements are :—

A. (*M.*) *gambiae* compared with *A.* (*S.*) *africanus*—$\chi^2=5{\cdot}9$ ($P<{\cdot}02>{\cdot}01$) ;
A. (*M.*) *gambiae* compared with *C. centurionis*—$\chi^2=0{\cdot}17$ ($P>{\cdot}1$) ;
A. (*S.*) *africanus* compared with *C. centurionis*—$\chi^2=0{\cdot}04$ ($P>{\cdot}1$).

Significant association occurs only in the *A.* (*M.*) *gambiae*—*A.* (*S.*) *africanus* pair in which there is distinct indication that an evening which yields above the mean of one of these species tends also to yield above the mean of the other and that evenings on which the catches are below the mean are similarly associated. In the other species pairs it may simply be noted that what little association is present is in the same direction.

The effects of weather on the catches of these three species may now be examined. The weather data in a few cases were incomplete and these evenings have been discarded. In all cases the weather conditions have been considered only during the time of high activity ; these times were :—

A. (*M.*) *gambiae*—Catch periods 6 to 12
A. (*S.*) *africanus*—Catch periods 5 to 12
C. centurionis—Catch periods 4 to 12

An evening has been considered as exhibiting a given weather condition if that condition occurred during any part of the time indicated.

A. (*M.*) *gambiae.* The occurrence of catches above and below the mean is compared with the prevalence of cloud, wind and rain in Table III. The values of χ^2 and the corresponding values of P for these associations are :—

Cloud	$\chi^2=0{\cdot}05$	$P>{\cdot}1$
Wind	$=7{\cdot}1$	$<{\cdot}01$
Rain	$=0{\cdot}96$	$>{\cdot}1$

Cloud and rain seem therefore to be without effect but the presence of wind is associated significantly with a diminished catch.

TABLE III.

The distribution of geometric mean catches of *A.* (*M.*) *gambiae* per platform for individual evenings above and below the same mean based on the three evenings centred on the evening in question ; compared with weather conditions.

Weather	Above the mean	Below the mean	Totals
Cloud ...	13	15	28
No cloud	25	32	57
Totals ...	38	47	85
Wind ...	6	20	26
No wind	35	30	65
Totals ...	41	50	91
Rain ...	3	8	11
No rain ...	39	42	81
Totals ...	42	50	92

A. (*S.*) *africanus*. Table IV gives similar comparisons for this species. The values of χ^2 and the corresponding values of P for these arrangements are :—

Cloud ... ... $\chi^2=3{\cdot}2$ ... $P<{\cdot}1>{\cdot}05$
Wind ... ... $=4{\cdot}5$ ... $<{\cdot}05>{\cdot}02$
Rain ... ... $=5{\cdot}5$ ... $<{\cdot}02>{\cdot}01$

The effect due to cloud cannot be considered significant. However, a probably significant result occurs in the comparison with windy evenings and an almost certainly significant one in that with rainy ones ; it may be concluded that wind and rain tend to diminish the size of the catch.

TABLE IV.

The distribution of geometric mean catches of *A.* (*S.*) *africanus* per platform for individual evenings above and below the same mean based on the three evenings centred on the evening in question ; compared with weather conditions.

Weather	Above the mean	Below the mean	Totals
Cloud ...	9	19	28
No cloud	29	26	55
Totals ...	38	45	83
Wind ...	9	20	29
No wind	33	27	60
Totals ...	42	47	89
Rain ...	1	10	11
No rain ...	41	38	79
Totals ...	42	48	90

C. centurionis. Table V makes similar comparisons for this species. The appropriate values for χ^2 and P are :—

Cloud ... ... $\chi^2=0{\cdot}02$ ... ... $P>{\cdot}1$
Wind ... ... $=1{\cdot}6$ ... ... $>{\cdot}1$
Rain ... ... $=1{\cdot}9$ ... ... $>{\cdot}1$

None of these weather conditions therefore shows significant association with the size of the catch of *C. centurionis*. It should be noted, however, that the proportion of above-mean catches is larger on windy than on windless evenings, *i.e.*, what effect is attributable to wind tends to be in the opposite direction to that obtaining in the cases of *A.* (*M.*) *gambiae* and *A.* (*S.*) *africanus*. Wind effect is almost certainly the main cause of the correspondence found between these two species and the lack of correspondence when either of them is compared with *C. centurionis*.

The biting rhythm.

The biting rhythm of the more numerous species was studied by computing the geometric mean catch per platform per night for each of the fourteen periods into which the catches were divided. The geometric mean biting rate per hour was then derived for each period by multiplying by the appropriate factor, *e.g.*, by 6 in the

TABLE V.

The distribution of geometric mean catches of *C. centurionis* per platform for individual evenings above and below the same mean based on the three evenings centred on the evening in question ; compared with weather conditions.

Weather	Above the mean	Below the mean	Totals
Cloud ...	16	13	29
No cloud	30	26	56
Totals ...	46	39	85
Wind ...	18	12	30
No wind	28	33	61
Totals ...	46	45	91
Rain ...	3	8	11
No rain ...	44	37	81
Totals ...	47	45	92

case of the 10-minute periods. The biting rhythm is given for each species on this basis and in the abundant species attempts have also been made to study differences due to platforms and the effects of season and weather. On twenty-seven evenings rain fell during the catch but only nine of these experienced rain during catch periods 7 to 12 inclusive, which include almost always the peak period of activity. All these nine evenings were mainly or entirely cloudy and only one was windy between catch periods 7 and 12. Twenty-four evenings were windy at some time during catch periods 7 and 12 inclusive ; four of these evenings were cloudy and one had rain.

There were 31 evenings on which the moon was above the horizon for the whole of the hour following sunset and on which the disc was more than 10 per cent. illuminated. They occurred between the 16th and 24th March, 13th and 15th April and 5th and 16th November 1948, on the 11th and 12th February and between the 4th and 14th March 1949. Other evenings are regarded as moonless.

In view of the complexity of the climatic changes it has been attempted here only to test if any wide differences from the general rhythm are shown by the evenings of special climatic conditions. The results for the various species over the whole catch series, for various subdivisions, and evenings of special climatic conditions are given in Tables VI and X. The more important curves are shown graphically in figs. 4 and 5.

A. (*M.*) *gambiae.* The peak of biting activity falls in catch period 9 whether the whole series, or subdivisions by platforms, catch groups or rainy, windy, moonlit or moonless evenings are considered (Table VI) ; fig. 4 shows the curves for the whole series and for rainy evenings.

Up to the end of catch period 4, no more than occasional specimens were taken and the beginning of the main activity period is usually indicated by a fairly sharp rise in the curve. Over the whole catch series this takes place in catch period 6 but there are some variations in the subdivisions (Table VI). In the case of platform subdivisions, the main activity began in catch period 5 (platform 4), 6 (platforms 3 and 5) or 7 (platforms 1 and 2). This would not appear to be a light effect dependent on the shading of the platform itself as platforms 1 and 2, on both of which activity

TABLE VI.

Geometric mean numbers biting per platform per hour in the 14 catch periods. Results are given for each species over the whole catch series of 100 nights and/or for subdivisions as numbers allow. The peak activity periods are *in italics.*

Species	Part of catch series included	Period of catch Sunset														Number of insects on which based
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
A. (*M.*) *gambiae*	All series	—	—	·01	·02	·03	·16	·31	1·19	*1·99*	1·48	·79	·69	·37	·45	1,771
	Platform 1	—	—	·01	·04	—	·04	·29	1·52	*3·20*	2·61	1·54	·93	·47	·67	520
	,, 2	—	—	—	—	—	·04	·45	1·26	*1·61*	1·01	·25	·60	·42	·38	290
	,, 3	—	·06	·04	—	—	·19	·14	·82	*1·87*	1·64	·88	·84	·36	·45	373
	,, 4	—	—	—	—	·11	·17	·40	1·39	*1·97*	1·42	·79	·74	·33	·51	363
	,, 5	—	—	·02	·07	·04	·15	·24	1·00	*1·43*	·97	·58	·35	·28	·30	225
	Group 1	—	—	—	—	—	·20	·17	·44	*·60*	·34	·11	·22	·27	·55	181
	,, 2	—	·08	·08	·08	·13	·28	·46	1·83	*3·73*	3·13	1·60	1·04	·73	·56	514
	,, 3	—	—	—	—	·04	·24	·69	2·38	*4·06*	3·55	1·79	2·09	·85	1·09	781
	,, 4	—	—	·04	—	—	·04	·20	1·45	*1·76*	·89	·40	·33	·13	·29	224
	,, 5	—	—	·04	·04	—	·08	·04	·16	*·66*	·29	·36	·04	·09	·05	71
	Rainy evenings ...	—	—	—	·15	·08	·25	·44	·60	*1·13*	·70	·80	·64	·35	·48	122
	Windy evenings ...	—	—	—	—	·04	—	·18	·51	*·80*	·45	·28	·33	·24	·52	235
	Moonlit evenings ...	—	—	·02	—	—	·02	—	·67	*1·58*	1·34	·83	·55	·38	·54	511
	Moonless evenings ...	—	—	—	·04	·04	·22	·44	1·44	*2·18*	1·53	·78	·76	·36	·42	1,260
T. (*C.*) *fuscopennatus* ...	Group 3	—	—	—	—	—	·04	·19	·39	1·19	*1·72*	1·07	·50	·14	·13	173

TABLE VI (continued).

T. (M.) africanus ...	Groups 1–3 ...	—	—	—	—	—	·03	—	·15	·55	·71	*·79*	·44	·25	·24	424
	Platform 1	—	—	—	—	—	·08	—	·32	1·01	1·13	*1·40*	·77	·38	·40	135
	,, 2	—	—	—	—	—	·08	—	—	·22	·96	*1·03*	·69	·39	·20	98
	,, 3	—	—	—	—	—	—	—	·14	·77	·77	1·09	·30	·28	·27	94
	,, 4	—	—	—	—	—	—	—	·30	·30	·42	*·50*	·34	·16	·25	64
	,, 5	—	·08	—	—	—	—	—	—	*·46*	·34	—	·14	·09	·11	33
	Group 1	—	·01	—	—	—	—	—	·09	*·48*	·31	·25	·22	·17	·21	89
	,, 2	—	—	—	—	—	—	—	—	·19	*·67*	·49	·33	·49	·67	63
	,, 3	—	—	—	—	—	·08	—	·35	·97	1·17	*1·65*	·77	·53	·41	272
	Windy evenings ...	—	·01	—	—	—	·01	—	—	·19	*·35*	·25	·19	·16	·30	87
T. (M.) uniformis ...	Groups 1–3 ...	—	—	—	—	—	·01	·03	·03	·24	*·49*	·31	·19	·08	·10	169
	Platform 1	—	—	—	—	—	—	—	·08	·14	·38	*·91*	·38	·14	·19	54
	,, 2	—	—	—	—	—	—	·08	—	·34	*·46*	·38	·14	·14	·08	38
	,, 3	—	—	—	—	—	·08	—	—	·30	*·91*	·14	·30	·06	·11	38
	,, 4	·08	—	—	—	—	—	·08	·08	·19	*·22*	·08	·08	·02	·08	20
	,, 5	—	—	—	—	—	—	—	—	·22	*·52*	·08	·08	·04	·04	19
	Group 1	—	—	—	—	—	—	·04	·04	·09	*·41*	·36	—	·04	·14	46
	,, 2	·01	—	—	—	—	—	—	—	·14	·19	*·41*	·14	·07	·02	32
	,, 3	—	—	—	—	—	·04	·04	·04	·48	*·86*	·22	·43	·13	·13	91
A. (M.) nigerrimus ...	Groups 1–3 ...	—	—	—	—	—	·03	·01	·04	·13	·12	·13	·08	·07	·11	125
A. (S.) apicoargenteus ...	Groups 1–3 ...	·12	·11	·05	·03	·01	·01	·04	—	·01	—	—	—	—	—	122

began late, were the least and the most shaded by foliage, respectively. The main activity began in catch period 5 in catch group 2, in period 6 in groups 1 and 3, in period 7 in group 4 and in period 8 in group 5. The onset of activity tends, then, to be later in catch groups falling in the drier, hotter weather. On rainy evenings, considerable activity occurred as early as catch period 4. The main period of biting activity on moonlit evenings began in period 8 and was thus the latest of any of the sub-divisions ; on moonless evenings, it started in period 6.

T. (*C.*) *fuscopennatus*. Analysis is confined to catch group 3 ; only 18 of the total of 191 specimens occurred in other groups. The peak of biting activity fell in catch period 10 (Table VI & fig. 4) ; analysis by separate platforms gave the same result except that on platform 4 the values for periods 9 and 10 were equal.

The onset of the main activity fell in catch period 7 (Table VI).

T. (*M.*) *africanus*. Analysis is confined to the first three catch groups ; only four of the total of 428 specimens taken occurred in other groups. The peak of biting activity fell in catch period 11 when all the data were taken together (Table VI & fig. 4). The several platforms gave the same result except in the case of platform 5 in which the peak fell in period 9 ; the latter result, however, is based on small numbers only. When the data were broken down by catch groups a progressive advancement of the peak period of activity was found ; it fell in catch period 9 in catch group 1, in period 10 in group 2 and in period 11 in group 3 (Table VI). The numbers of mosquitos on which this finding is based, for periods 9 to 11, are given in Table VII ; χ^2 for this arrangement is 11·7 and P is near ·02, *i.e.*, significant. Only 14 specimens were taken on the nine rainy nights ; the effect of rain has therefore not been studied. On windy nights the peak of biting activity was very poorly marked but was still evident in catch period 10 (Table VI & fig. 4).

TABLE VII.

The total numbers of *T.* (*M.*) *africanus* occurring in catch periods 9, 10 and 11 in the three catch groups 1, 2 and 3.

Catch period	9	10	11	Totals
Group 1	12	7	6	25
Group 2	4	15	11	30
Group 3	25	30	45	100
Totals	41	52	62	155

The period of main activity began in catch period 8 or 9 (Table VI).

T. (*M.*) *uniformis*. Analysis is confined to the first three catch groups as the species did not occur in the others. The peak period of biting activity fell in catch period 10 over the whole data and also in the cases of the several platforms, with the exception that it was delayed one period on platform 1 (Table VI). The numbers of specimens concerned in this finding, for periods 10 and 11, are given in Table VIII ; the value of χ^2 is 11·4 and hence $P < \cdot01$, *i.e.*, highly significant. It may be accepted therefore that the time of peak biting activity on platform 1 was slightly later than on the others ; platform 1 was the least shaded one. When the data are analysed by groups, the peak falls in catch period 10 in groups 1 and 3, in period 11 in group 2. The numbers of specimens here concerned are given in Table IX ; the adjusted value of χ^2 for this arrangement is 5·6 and hence $P < \cdot05 > \cdot02$, *i.e.*, probably significant.

The beginning of the time of main activity occurred with only one exception in catch period 9. The numbers taken are considered too small for any study of weather effects.

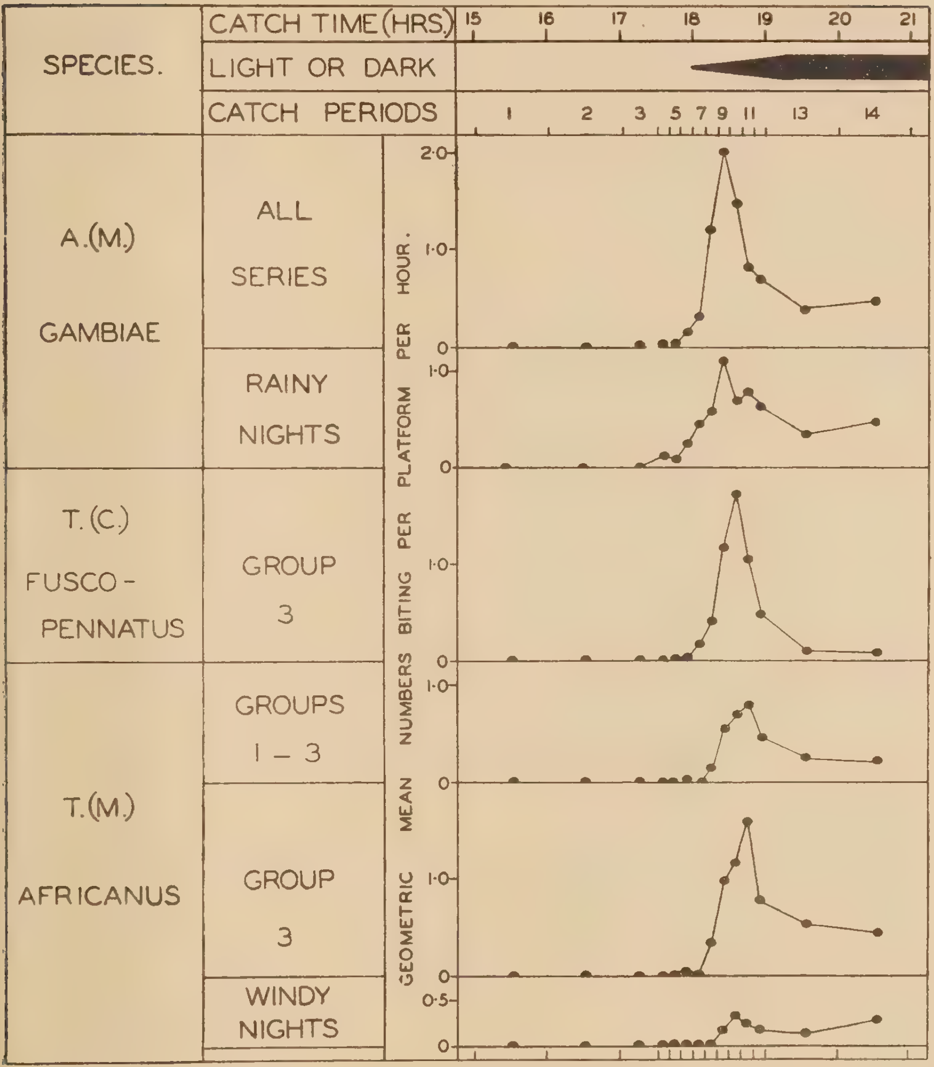

Fig. 4.—Representative biting activity curves for *A.* (*M.*) *gambiae*, *T.* (*C.*) *fuscopennatus* and *T.* (*M.*) *africanus*. For method of calculation see text. The tip of the black wedge coincides with the time of sunset, its base with that of Astronomical Twilight.

TABLE VIII.

The total numbers of *T.* (*M.*) *uniformis* occurring in catch periods 10 and 11 on platform 1 and platforms 2 to 5.

Catch period	10	11	Totals
Platform 1	5	12	17
Platforms 2–5	30	9	39
Totals	35	21	56

TABLE IX.

The total numbers of *T.* (*M.*) *uniformis* occurring in catch periods 10 and 11 in catch groups 1 and 3, and 2.

Catch period	10	11	Totals
Catch groups 1, 3	31	12	43
Catch group 2	4	9	13
Totals	35	21	56

A. (*M.*) *nigerrimus.* Only 126 specimens were taken, all except one in catch groups 1–3, and no attempt has been made to subdivide the data. There is a poorly-marked period of higher biting activity in periods 9–11 (Table VI & fig. 5).

A. (*S.*) *apicoargenteus.* Only 10, of the total of 132 of this species taken, occurred in groups 4 and 5; these groups have therefore been discarded and the analysis relates to 122 specimens taken in groups 1 to 3. The number being small, subdivision has not been attempted. Biting activity is diminishing through the early periods and the latest specimen taken biting was in catch period 9 (Table VI & fig. 5).

A. (*S.*) *africanus.* The peak of biting activity fell in catch period 9 over the whole series (Table X & fig. 5). It occurred in this period also in the subdivisions by platforms with the exception of platform 5 on which it was in period 7; Table XI shows the relevant numbers and for the comparison of platform 5 with the others, χ^2 is 16·9 and hence $P<\cdot01$, *i.e.*, highly significant. The peak of biting activity fell in catch period 9 in catch groups 1, 3 and 4, in period 8 in group 2 and in period 7 in group 5. Table XII shows the numbers of mosquitos concerned and, for comparisons of group 2 and group 5 in turn with the remainder taken together, the χ^2 values are, respectively, 11·2 ($P<\cdot01$) and 8·8 ($P<\cdot02>\cdot01$), *i.e.*, highly significant and significant. Catch period 9 included the peak of biting-activity on windy, rainy, moonlit and moonless evenings (Table X).

Over the whole series the main biting activity began in period 5. It was the same on the several platforms except for platforms 3 and 4 on which it began in periods 4 and 6, respectively. The main biting activity began earliest in catch groups 2 (period 3) and 1 (period 4); in groups 3 to 5 it began in period 7. The main biting activity began in period 7 on the rainy evenings, in period 5 on the windy ones; it tended to begin later on the moonlit evenings (period 7) than on the moonless ones (period 6) (Table X).

It may be noted that the platform which showed the early peak activity did not also show an early activity onset. Nor was there parallel variation of these two episodes in the division by catch groups.

Chrysops centurionis. The peak of activity over the whole series fell in catch period 8 (Table X & fig. 5). Analysing the data by platforms the same result was found except for platforms 2 and 4 on which the peak fell one period later and earlier, respectively. Table XIII shows the numbers of specimens concerned and for comparisons of these two aberrant cases with the remainder the χ^2 values are respectively, 1·2 ($P>\cdot1$) and 1·7 ($P>\cdot1$); these differences are therefore not considered significant. The peak of biting activity is retarded by one period in catch groups 3 to 5 as compared with groups 1 and 2. Table XIV shows the relevant numbers of specimens; the value of χ^2 is 8·6 and hence $P<\cdot01$. *i.e.*, highly significant. Catch groups 1 and 2 cover a period of exceptional abundance of *C. centurionis* (p. 731); during this time it appears, then, that the species tends to bite earlier than in the other catch groups. The peak of biting activity fell in period 9 on the rainy evenings, in period 8 on the

TABLE X.

Geometric mean numbers biting per platform per hour. Results are given for each species over the whole catch series of 100 evenings and/or for subdivisions as numbers allow. The peak activity periods are in italics.

Species	Part of catch series included	Period of catch														Number of insects on which based
							Sunset									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
A. (S.) africanus	All series	·02	·01	·04	·08	·20	·32	·55	1·08	*1·28*	·71	·29	·22	·11	·07	841
	Platform 1	·01	·01	·05	—	·15	·17	·56	1·33	*1·36*	·86	·65	·35	·13	·12	193
	,, 2	·04	·04	·02	·08	·48	·53	·79	1·73	*1·85*	·71	·19	·30	·16	·09	236
	,, 3	·03	·01	·06	·19	·11	·45	·41	1·19	*1·82*	1·26	·48	·21	·13	·07	220
	,, 4	·04	—	·04	·04	·04	·17	·28	·61	*·92*	·51	·08	·16	·08	·05	95
	,, 5	·03	—	·06	·08	·21	·31	*·74*	·60	·56	·28	·08	·08	·03	·04	97
	Group 1	·05	·02	·07	·20	·56	·82	1·20	1·30	*1·54*	1·27	·23	·44	·21	·27	282
	,, 2	·04	·04	·14	·13	·25	·67	·95	*2·73*	2·02	·85	·52	·37	·15	·05	281
	,, 3	·01	·01	—	·08	·08	·08	·37	1·55	*2·82*	1·48	·63	·13	·13	·08	226
	,, 4	—	·04	—	—	·04	—	·15	·11	*·37*	·08	—	·15	·05	·01	34
	,, 5	—	—	—	—	·08	—	*·19*	·08	·08	·08	·12	·01	·01	—	18
	Rainy evenings ...	·03	—	·06	—	—	—	·28	·28	*·44*	·08	·08	—	·01	·03	20
	Windy evenings ...	·02	—	·02	·05	·32	·33	·51	·79	*·80*	·41	·04	·29	·16	·17	187
	Moonlit evenings ...	·02	·02	·06	·10	·19	·11	·48	·92	*1·33*	·94	·52	·26	·12	·10	278
	Moonless evenings ...	·02	·01	·03	·07	·20	·43	·58	1·15	*1·27*	·62	·19	·20	·10	·06	563
Chrysops centurionis ...	All series	·07	·07	·15	·46	·54	·91	1·35	*1·74*	1·54	1·28	1·06	·79	·27	·11	1,946
	Platform 1	·03	·05	·06	·28	·44	1·14	1·61	*2·72*	2·20	1·52	1·88	1·15	·26	·11	526
	,, 2	·14	·12	·20	·62	·60	·97	1·37	1·48	*1·54*	·94	1·01	·80	·27	·08	370
	,, 3	·12	·11	·28	·67	·89	1·02	1·52	*1·96*	1·81	1·82	·84	·90	·32	·16	519
	,, 4	·01	·04	·09	·22	·17	·59	*1·08*	·98	·87	·87	·77	·50	·22	·09	224
	,, 5	·04	·04	·11	·49	·64	·82	1·17	*1·66*	1·33	1·33	·85	·59	·25	·13	307
	Group 1	·15	·18	·31	1·13	1·28	2·47	4·01	*5·78*	4·33	4·01	3·49	2·05	·82	·35	1,077
	,, 2	·16	·18	·37	·95	·92	1·19	2·32	*2·74*	1·48	1·53	1·09	·89	·30	·14	451
	,, 3	·04	—	·03	·13	·25	·24	·32	·52	*·65*	·38	·47	·43	·11	·06	118
	,, 4	—	·02	·03	·11	·29	·70	·60	·94	*·98*	·62	·41	·37	·14	·03	165
	,, 5	·02	·01	·08	·16	·14	·31	·45	·31	*·95*	·68	·47	·45	·13	·03	135
	Rainy evenings ...	·11	·07	·48	·54	·28	·25	·28	·60	*·80*	·44	·21	·21	·16	·04	90
	Windy evenings ...	·04	·08	·16	·66	·88	1·48	2·61	*3·55*	2·72	2·66	2·20	1·38	·43	·24	873
	Moonlit evenings ...	·04	·05	·05	·11	·43	·52	·97	1·21	*1·37*	·87	·92	·58	·23	·07	420
	Moonless evenings ...	·08	·08	·19	·62	·59	1·09	1·52	*1·99*	1·61	1·49	1·13	·88	·28	·13	1,526

windy ones (Table X). There was apparently a retardation of the peak period of biting activity by one period on the moonlit evenings as compared with the moonless ones (Table X). However, the numbers taken in periods 8 and 9 were, respectively, on moonlit evenings, 53 and 59, on moonless evenings, 210 and 168 ; χ^2 is 2·4 and $P > \cdot 1$, the difference being therefore not considered significant.

TABLE XI.

The total numbers of *A.* (*S.*) *africanus* occurring in catch periods 7, 8 and 9 on platforms 1 to 4 and 5.

Catch period	7	8	9	Totals
Platforms 1–4	51	146	164	361
Platform 5	19	16	16	51
Totals	70	162	180	412

TABLE XII.

The total numbers of *A.* (*S.*) *africanus* occurring in catch periods 7, 8 and 9 in catch groups 1, 3 and 4, and 2 and 5.

Catch period	7	8	9	Totals
Catch groups 1, 3, 4 ...	41	83	120	244
Catch group 2	24	81	56	161
Catch group 5	5	2	2	9
Totals	70	166	178	414

TABLE XIII.

The total numbers of *C. centurionis* occurring in catch periods 7, 8 and 9 on platforms 1, 3 and 5, 2 and 4.

Catch period	7	8	9	Totals
Platforms 1, 3, 5 ...	142	193	163	498
Platform 2	39	41	42	122
Platform 4	28	29	22	79
Totals	209	263	227	699

TABLE XIV.

The total numbers of *C. centurionis* occurring in catch periods 8 and 9 in catch groups 1 and 2 and 3 to 5.

Catch period	8	9	Totals
Catch groups 1, 2	217	162	379
Catch groups 3, 4, 5 ...	46	65	111
Totals	263	227	490

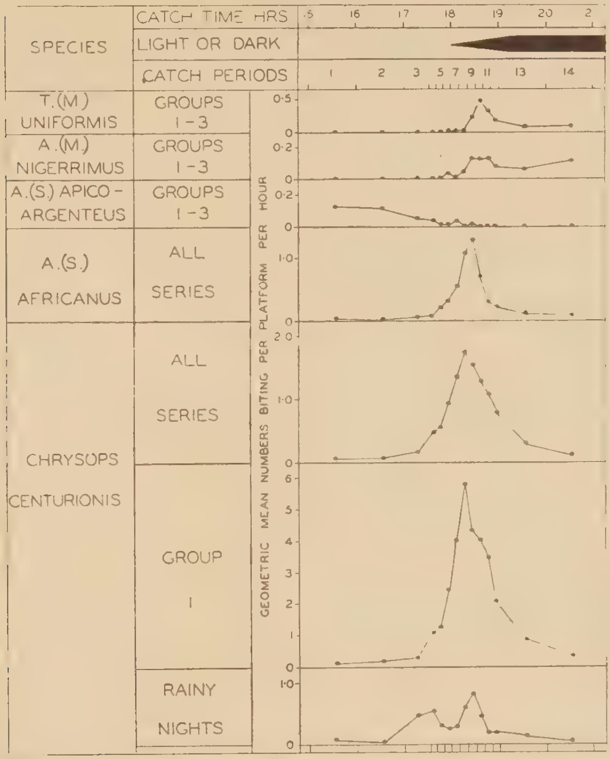

Fig. 5.—Representative biting activity curves for *T.* (*M.*) *uniformis*, *A.* (*M.*) *nigerrimus*, *A.* (*S.*) *apicoargenteus* and *africanus* and *C. centurionis*. For method of calculation see text. The tip of the black wedge coincides with the time of sunset, its base with that of Astronomical Twilight.

The beginning of the main biting activity is difficult to define clearly but is generally in catch period 4 ; it was earlier in catch groups 1 and 2 and on rainy evenings ; it was also earlier on moonless than on moonlit evenings.

Individual evenings.—The foregoing studies deal with considerable numbers of catches taken together, a procedure which in most species is necessary in order to procure the numbers required to define the curves of biting activity ; they are therefore concerned with the average curves over considerable periods. In some species, however, numbers sufficient to define a curve are available for several single evenings and in these cases it is possible to study what variation takes place from day to day. Such study is mainly devoted to the time of peak activity as this is most easy to locate. It may be defined as the single value, or adjacent pair of equal values, which is the maximum, the two values before and after it either successively diminishing or equal or nearly equal to each other. Selected data are shown in Table XV and graphically in fig. 6.

A. (*M.*) *gambiae*. There was no difference in the period of peak activity between platforms over the whole series so that all platforms have been taken together to give geometric mean numbers biting per hour per platform for all the periods for a single evening's catch. There were twenty-seven evenings on which the total catch of *A.* (*M.*) *gambiae* was 20 or over ; 23 of these yield curves showing clearly-marked peaks of biting activity. Three typical sets of data based on single days are given in

TABLE XV.

Geometric mean numbers biting per platform per hour. Selected individual nights for some of the more numerous species. The peak activity periods are in italics.

Species	Date	Period of Catch														Number of insects on which based
		1	2	3	4	5	Sun 6	set 7	8	9	10	11	12	13	14	
A. (*M.*) *gambiae* ...	25 November 1948	—	—	—	—	—	3·86	*20·6*	6·53	5·97	3·51	1·91	3·08	·98	·43	64
	6 November 1948	—	—	—	—	—	—	—	7·01	*26·8*	19·7	10·8	6·31	2·39	2·77	116
	2 November 1948	—	—	—	—	—	—	—	4·19	15·9	*18·3*	16·3	8·66	2·57	2·53	123
	29 April 1948 ...	—	—	—	—	—	—	·89	5·43	1·91	7·01	·89	9·64	2·25	1·12	52
T. (*M.*) *africanus* ...	16 November, 1948	—	—	—	—	—	—	—	·89	·89	·89	*3·86*	·89	·30	·74	25
A. (*S.*) *africanus* ...	3 April 1948 ...	—	—	—	—	6·31	*7·31*	1·48	—	—	—	—	—	—	—	20
	27 April 1948 ...	·15	—	—	—	·89	·89	3·08	*5·86*	3·51	·89	·89	—	·32	—	23
	12 November 1948	—	—	—	—	—	—	—	1·91	*6·31*	5·33	·89	—	·15	·32	20
	4 May 1948 ...	—	—	·64	·89	·89	3·86	1·48	6·53	3·34	—	·89	·89	·06	·06	26
Chrysops centurionis	9 April 1948 ...	·51	·43	*3·93*	—	—	—	—	1·48	—	—	—	—	—	—	26
	26 March 1948 ...	—	·51	·86	—	·89	4·72	18·6	*31·7*	13·7	8·06	7·31	7·01	·89	·89	128
	18 March 1948 ...	·15	·15	1·03	—	—	·89	·89	1·48	2·59	*4·72*	3·86	2·59	·46	1·05	45

Table XV and show peaks of biting activity in periods 7, 9 and 10 respectively (fig. 6). Of the total of 23 nights on which the period of peak activity was clearly defined the distribution of the peak periods was :—

Period 7	1
7 and 8 equal	1
8	1
9	17
10	3

The times of the occurrence of the period of peak activity are compared with weather conditions in Table XVI ; while these numbers are too unevenly distributed for the application of satisfactory tests of significance there does appear to be an effect due to cloud, 2 out of 3 of the early, about half of the intermediate, and 1 out of 3 of the late peaks were on cloudy days.

One of the catches which did not produce a clearly defined single peak of activity is also shown in Table XV ; this particular day (29th April 1948) was cloudy only during catch periods 1 to 3 and there was no wind or rain during the catch. Of the three other catches in which the peak was not defined, one was cloudy throughout, windy up to period 5, and rainy up to period 11 and the other two were clear and fair with wind, in one case, between periods 3 and 9 and in the other in the first catch period only.

TABLE XVI.

The time of the occurrence of the peak biting activity in *A.* (*M.*) *gambiae* compared with weather conditions.

Catch periods	7–8	9	10	Totals
Times peak biting activity occurred	3	17	3	23
Times cloud occurred in catch periods 5–9	2	8	1	11
Times wind occurred in catch periods 5–9	0	4	0	4
Times rain occurred in catch periods 5–9	1	0	0	1

T. (*M.*) *africanus*. The total catch of this species over the five platforms exceeded 20 only six times, all in catch group 3. The biting rhythm on these six occasions was examined after the elimination of platform 5 which may be different from the others (p. 738) and in any case produced only small numbers. The peak of biting activity for this species in catch group 3, all platforms being taken into account, fell in catch period 11 (p. 738). A typical example, given in Table XV, and fig. 6, shows the peak of activity falling also in catch period 11. In the total of six individual evenings examined the peak of biting activity fell once in period 10, four times in period 11 and in one case was not clearly defined.

A. (*S.*) *africanus*. Neglecting the data of platform 5 on which the period of peak activity was found to occur (p. 740) slightly earlier than on the others, there were thirteen evenings on which the total catch amounted to 20 specimens or over. Of these, 11 show clearly-defined single activity peaks which occurred as follows :—

Catch period 6	1
7 and 8 equal	1
8	5
9	2
9 and 10 equal	1
11	1

Three characteristic curves (3rd April, 27th April and 12th November 1948) are given in Table XV and fig. 6. None of these eleven evenings was rainy except on two occasions in the last two catch periods. Of the seven evenings on which the peak was early (catch periods 6 to 8) two were cloudy and two windy between periods 5 and 9. Of the four evenings on which the peak was late (periods 9 to 11) one was windy and one cloudy during the same interval. The two remaining evenings show a mean peak of biting activity in periods 9 and 8 respectively, with a subsidiary peak, nearly approaching the main peak in magnitude, in periods 11 and 6 respectively. Both these evenings were clear, fair and windless during periods 5 to 9; the data for one of them (4th May 1948) is shown in Table XV.

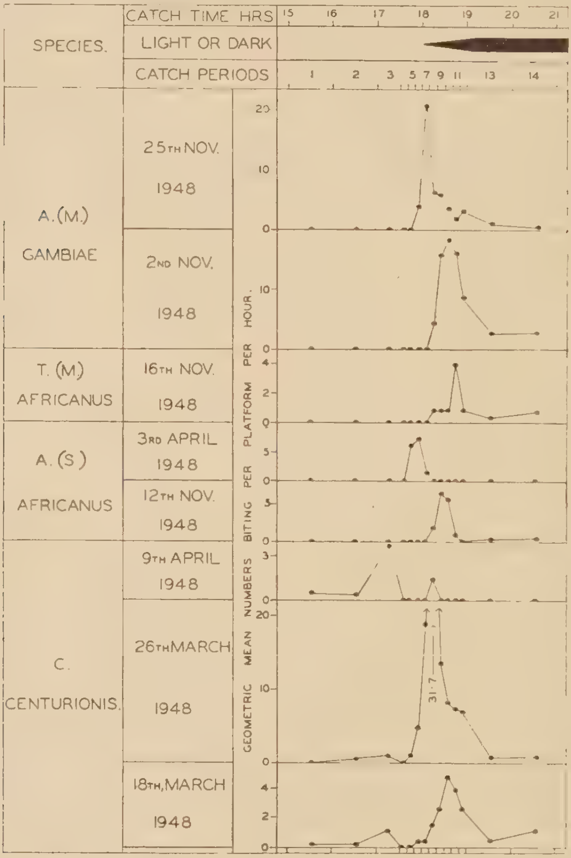

Fig. 6.—Representative biting activity curves, based on individual evenings, for *A.* (*M.*) *gambiae*, *T.* (*M.*) *africanus*, *A.* (*S.*) *africanus* and *C. centurionis*. For method of calculation see text. The tip of the black wedge coincides with the time of sunset, its base with that of Astronomical Twilight.

Chrysops centurionis. No significant differences were found between the platforms (p. 740) so all have been included in the study of individual evenings. There were 26 evenings on which the total catch exceeded 19 specimens. Three typical sets of data showing early (9th April 1948), intermediate (26th March 1948) and late (18th March, 1948) peaks are given in Table XV and fig. 6. It will be observed that all these three curves are bimodal, showing an early peak, in one case (9th April 1948) the larger, in period 3 and a later one, in the other two cases the larger, in period 8 or 10. Such a separation of the curve into two portions is shown clearly on twelve of the twenty-six individual evenings under study ; the early peak period of activity fell as follows :—

Catch period 1	1
2	1
2 and 3 equal	1
3	3
4	4
5	2

Nine other evenings show some indication of a like occurrence but the numbers are small. The remaining five yielded no specimens in the first four or five periods. The occurrence of the phenomenon of an early peak may be compared with the dates of the catches. The period of greatest abundance of *C. centurionis* was between the 17th March and 10th April 1948 (p. 731). The distribution of catches yielding unimodal and bimodal curves and curves indefinitely of either type is shown in Table XVII. Application of the adjusted χ^2 test to the distributions of the definite curves only, gives a value of 9·3 and hence $P<\cdot 01$, *i.e.*, highly significant. Bimodal curves are therefore most likely to occur during the period of maximum abundance of the species. This finding is also reflected in the data given for *C. centurionis* by catch groups in Table X ; considerable values occur in the first five catch periods in catch groups 1 and 2 but not in catch groups 3 to 5.

TABLE XVII.

The occurrence of unimodal and bimodal curves of biting activity and of curves indefinitely of either type for *C. centurionis* on the 26 nights on which the total catch exceeded 19. For explanation see text.

Date of catch	Type of curve			Totals
	Unimodal	Bimodal	Indefinite	
17 March to 10 April 1948	0	11	6	17
Other dates	5	1	3	9
Totals	5	12	9	26

Confining attention to the second peak of activity only, in the 26 nights available it was found that 20 nights yielded clearly-defined peaks. These were distributed as follows :—

Catch period 7	4
8	10
9	1
9 and 10 equal	1
10	3
11	1

Of the 14 nights on which the peak was early (in catch periods 7 and 8) three were cloudy, eight were windy and one was rainy during catch periods 6 to 10. Of the six nights on which the peak was late (catch periods 9 to 11), two were cloudy, five were windy and none was rainy during catch periods 6 to 10. Here again the numbers are too small to show clearly any influence of weather but there is perhaps an effect due to wind, its presence tending to delay the time of peak activity.

There were seven evenings on which *A.* (*M.*) *gambiae* and *A.* (*S.*) *africanus* each yielded catches exceeding 19. Similarly there were for the pairs *A.* (*M.*) *gambiae* and *C. centurionis* and *A.* (*S.*) *africanus* and *C. centurionis*, respectively, two and five such evenings. The advancement or retardation of the peak period of biting activity was compared in these catches between pairs of species but no consistent result was obtained.

The less abundant Species.

Species groups are also included here. The crude results are summarised in Table XVIII. In most cases the numbers are too small for serious consideration but a few comments may be made.

All the *Anopheles* spp. occurred after sunset and almost all in the catch groups which fell in the wet weather.

The species of *Taeniorhynchus* were mainly active after sunset and, with the exception of two *T.* (*C.*) *aurites*, were confined to catch group 3 thus closely resembling the findings in the three commoner species of the genus discussed above.

Aëdes (*F.*) *longipalpis* was confined to the first two catch periods and to the two wettest catch groups. *A.* (*F.*) *ingrami* showed some activity in later periods, about the time of sunset.

A. (*A.*) *natronius* was confined to catch group 4, that with the driest weather, when also the single specimen of *Aëdomyia africana* occurred. *Aëdes* (*D.*) *furcifer*, with which species *A.* (*A.*) *natronius* has sometimes previously been confused (p. 724), was most frequent in the wet-season catch groups (2 and 3).

The undetermined species of *Culex* reached their greatest abundance in catch group 4, the driest one. They show a well-marked peak of biting-activity (on crude numbers) in the crepuscular period, in catch period 8.

Tabanids were taken mainly during the dry weather of catch groups 4 and 5.

The occurrence of *Glossina palpalis fuscipes* in the forest canopy is of interest. Only one of the total of seven taken was taken during the wetter catch groups 2 and 3. Haddow (personal communication) has taken *Glossina* spp. in forest catches of similar type at Bwamba but only at ground level. However, most of his work was done during the wet season and with the counter-attraction, absent in the present work, of a ground level team catching simultaneously.

Discussion.

Lumsden (1951a) found at Mongiro, Bwamba, that most black mangabeys (*Cercocebus albigena johnstoni* Lydekker), baboons (*Papio doguera tessellatus* Elliot) and lowland colobus monkeys (*Colobus abyssinicus ituricus* Matschie) had taken up their sleeping positions in the trees by sunset ; practically all had done so by ten minutes after sunset. The present type of catch, on a bait of boys sitting on a platform in the forest canopy, is therefore considered representative of the insect attack on sleeping monkeys, at least after sunset. It should, however, be noted that the attractiveness of man to biting insects is known not to be identical with that of monkeys (Haddow & Dick, 1948) and that the platforms are probably more representative of the sleeping positions adopted by baboon than by the other monkey species (Lumsden *loc. cit.*). In most of the previous work on forest insects in Bwamba, a bait of native boys was offered simultaneously at ground level and at various levels in the trees above. The absence, in the present study, of a counter-attracting bait catching at ground level may be of some importance ; for instance, the appearance of *Glossina palpalis fuscipes* may have been prevented in previous catches by the simultaneous offering of a bait at a preferred level. However, the present method, with no team

TABLE XVIII.

The occurrence of the less common species and of species groups in 100 evening catches at Mongiro, Bwamba ; March 1948 to March 1949.

Species or Group	Catch period														Catch group					Totals
						Sunset														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	
Anopheles coustani var. *ziemanni*	—	—	—	—	—	—	—	—	1	—	1	—	—	—	—	—	2	—	—	2
,, *implexus*	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	—	—	—	1
,, *funestus*	—	—	—	—	—	—	—	—	1	—	—	—	—	1	—	2	—	—	—	2
,, *demeilloni*	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	—	—	—	—	1
Aëdomyia africana	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1	—	1
Taeniorhynchus (*C.*) *metallicus*	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	—	—	1
,, *maculipennis*	—	—	—	—	—	—	—	—	1	1	1	—	3	—	—	—	6	—	—	6
,, *aurites*	—	—	—	—	—	1	—	—	1	—	1	—	—	1	1	1	2	—	—	4
,, *microannulatus*... ...	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1	—	—	1
Aëdes (*M.*) *grahami*	—	—	—	—	—	—	—	—	1	—	—	—	6	3	1	4	5	—	—	10
,, (*F.*) *longipalpis*	5	4	—	—	—	—	—	—	—	—	—	—	—	—	—	6	3	—	—	9
,, ,, *ingrami*	—	1	2	—	1	1	1	1	—	—	—	—	—	—	2	4	1	—	—	7
,, (*S.*) *simpsoni*	—	—	—	—	1	—	1	—	—	—	—	—	—	—	—	1	1	—	—	2
,, ,, *fraseri*	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1
,, ,, *luteocephalus*	—	—	—	—	—	—	—	—	—	—	2	—	—	—	1	—	1	—	—	2
,, (*A.*) *tarsalis* group	—	—	—	—	—	—	—	1	—	1	—	—	1	—	—	1	1	—	1	3
,, ,, *abnormalis* group	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	2	—	—	—	2
,, ,, *natronius*	—	—	—	—	—	—	1	—	—	—	1	—	2	—	*	—	—	4	—	4
,, (*D.*) *furcifer*	—	—	—	—	1	—	—	2	3	2	—	—	—	—	*	4	3	—	1	8
Culex (*C.*) *poecilipes*	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	—	—	1
Culex spp. indet.	2	—	2	2	5	8	25	39	27	14	15	11	29	24	38	20	34	91	20	203
Chrysops griseicollis	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1
Haematopota guineensis	1	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1	1	2
Tabanus par	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
,, *socialis*	—	—	1	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	2	2
,, *taeniola*	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	—	—	—	1
Glossina palpalis fuscipes	3	1	—	—	1	1	—	1	—	—	—	—	—	—	1	—	1	1	4	7

* Means no record.

present at ground level, imitates as closely as possible the conditions under which monkeys in the forest canopy are exposed to the attacks of biting insects and the catches are thus of importance in the consideration of the transmission of yellow fever virus and other parasites among forest monkeys.

Although the five platforms studied were close together and highly similar in their obvious characteristics, considerable differences appeared in the numbers of a given species taken on the different platforms. The platforms which produced the most and the least were, respectively, platforms 1 and 5 for *A.* (*M.*) *gambiae*, *T.* (*M.*) *africanus* and *uniformis*, platforms 2 and 4 for *A.* (*S.*) *apicoargenteus* and *africanus* and platforms 1 and 4 for *C. centurionis*. These differences cannot be correlated with any of the obvious characteristics of the platforms themselves, such as height, shading or sheltering by foliage. They are, however, nearly parallel in groups of species with similar breeding habit and are therefore concluded to be due probably to local differences in the numbers of the species present which in turn are almost certainly to be related to differences in the local abundance and proximity of breeding places. This hypothesis is supported by findings relating to *C. centurionis*. Considerable evidence is presented to show that this species hatches from the pupa largely, if not entirely, in late March and early April each year so that, if the hypothesis is true, it would be expected that differences between the platforms would be much more marked at this time than at other times in the catch series. When the results from all the platforms are tested statistically, significant differences are found both during the presumed hatching period and during the rest of the series but in the latter case the difference is almost entirely due to one platform (platform 4). If this platform is eliminated the catches from the remaining four still show wide and statistically highly significant differences during the presumed hatching period but at other times they are remarkably uniform and are not statistically different. The hypothesis is thus supported strongly by four out of five platforms and in the aberrant case a difference in the efficiency of the platform itself may be indicated ; platform 4 was situated at the base of a narrow neck of forest and thus perhaps tapped a smaller area of forest than did the others. However, whatever may be the causation of platform differences, it is clearly of advantage, in assessing the exposure of monkeys to attack by biting insects in the forest canopy, to sample several locations.

Seasonal variations in numbers were found to be of large amplitude and are considered to be almost certainly related mainly to the amount and distribution on rainfall affecting the profusion of breeding places at least as far as the mosquito species are concerned. In the case of species breeding in or in association with the nearby swamps (*A.* (*M.*) *gambiae*, *Taeniorhynchus* spp. and *A.* (*M.*) *nigerrimus*) the fluctuations in numbers show some similarities and the highest numbers of all these species occurred near the end of the wet season. In the case of species breeding in tree holes (*A.* (*S.*) *apicoargenteus* and *africanus*), the numbers taken at the end of the wet season were less than earlier in its course, an effect which Mattingly (1949b) has also noted in Nigeria, considering that high rainfall, by washing out the tree holes, may be a main factor in its causation.

The extent of the reduction in numbers during the dry season is of importance in relation to the survival of yellow fever virus in the forest. Smithburn & others (1949) came to the conclusion from a study of a sylvan outbreak of yellow fever in Bwamba that *A.* (*S.*) *africanus* was there the main insect host of the virus and it is generally agreed that a mammal cannot act as a reservoir over long periods owing to the transience of the typical peripheral blood infection. In the present series of catches two successive dry seasons are compared. In the second, though it was only slightly more rigorous as regards drought than was the first, there was a much more marked reduction in the numbers of biting insects. The numbers of all the main species reached their lowest levels in the dry weather of 1949. In the case of *A.* (*S.*) *africanus* evidence is brought to show that this reduction in numbers is a true decrease in population and not simply an effect of variation in activity. Its numbers were not seriously reduced in the first dry season but in the second it is estimated that under the slightly more rigorous conditions they fell to well under a twentieth of those existing at the end of the preceding wet season. Such an effect in a moderately rigorous dry season in the favourable forest environment suggests that considerably more severe effects may obtain in the northern and eastern part of Uganda where the dry season is much more severe. Evidence that this is so has been brought forward by Lumsden and Buxton (1951) for West Nile District where the dry season, though considerably more severe than in Bwamba, is by no means the most extreme example to be found in Uganda. It is known that in West Nile District at least, despite the more severe annual drought, yellow fever in monkeys is not uncommon (Lumsden & Buxton, 1951).

Gordon & others (1948) record that at Kumba in the British Cameroons the height of the *Chrysops* season is in June and July ; *C. silacea* and *C. dimidiata* were the only species taken biting and *C. silacea* was much the more abundant though *C. dimidiata* was stated to increase in numbers " towards the end of the rains ". Although considerable parts of the year in Bwamba are left uncovered by the present catch series there is no evidence of an outbreak of *C. centurionis* at all comparable to that demonstrated in late March and early April, 1948 ; the latter part of a similar episode was found by Haddow (private communication) at about the corresponding time in 1945. It seems reasonable to conclude that this is a yearly occurrence in Bwamba in March and April and is thus apparently considerably earlier than the main outbreak at Kumba. Evidence produced from the present work that during the outbreak the population consists of two separate groups is most simply explained by the feeding habits of newly-hatched females taking their first blood meal differing from those feeding on the second or subsequent occasions. Buxton (1950) has already expressed the belief that Tabanids have a sharply-defined season of emergence not only in deciduous woodland areas in Africa but perhaps also in tropical rain forest. Gordon & others (1948, 1950) discuss the breeding places of *Chrysops* spp. in West Africa and the stream described and illustrated as a typical breeding place bears a close resemblance to the lower parts of the streams and seepages in the forest at Mongiro, Bwamba. They record (1948) the finding of only three pupae in the course of extensive larval collections which suggests that pupation takes place in other locations, perhaps in the drier mud higher up the stream banks, or that the pupal stage is limited to a short period each year. The mechanism of the initiation of hatching, which in Bwamba apparently began a little before the onset of the main wet season, may have been related to the few isolated heavy rainstorms which preceded its opening. However, the general implications which follow from the finding, that between the yearly outbreaks the population of *C. centurionis* consists mainly of survivors from the previous hatching season, with probably only small additions by eclosion at other times, and therefore that the life of an individual female may approach a year in duration, are of significance in the consideration of the biology of the closely-allied species known to be vectors of loiasis to man.

Evidence is produced to show that the biting activity of *A.* (*M.*) *gambiae* was depressed by wind and that that of *A.* (*S.*) *africanus* was depressed by both wind and rain. *C. centurionis*, on the other hand, seemed little influenced by either of these factors and indeed what effect was produced by wind was in the opposite direction to that on the two mosquito species, *i.e.*, its numbers tended to increase on windy evenings. In the two mosquito species, the reduction of biting-activity associated with wind is to be compared with Johnson's (1950) work on the estimation of the aerial density of insects by various kinds of traps ; he states " the aerial density of insects usually changes in inverse order to the wind speed ". It is interesting to find that wind also affects in this way mosquitos ready to take a blood meal ; it might have been expected that insects locating their hosts mainly by olfactory organs would have been more efficient in the presence of some air movement. *C. centurionis*, a larger insect, is apparently unaffected by wind speeds sufficient to inhibit the mosquitos. The tendency for it to increase on windy nights, though not statistically significant, perhaps indicates that its olfactory sense plays some part in locating its prey.

Study of the changes in biting activity of insects of the forest canopy in catches which were divided into ten-minute periods over the critical interval between half an hour before and one hour after sunset revealed that all the species which yielded sufficient numbers for analysis and whose activity was known to be mainly nocturnal showed a peak of biting activity shortly after sunset which was followed by a fall in activity to moderate or low levels. This effect was found to be well marked in *A.* (*M.*) *gambiae*, *T.* (*C.*) *fuscopennatus*, *T.* (*M.*) *africanus*, *A.* (*S.*) *africanus* and *C. centurionis*, less so, but still distinct, in *T.* (*M.*) *uniformis* and *A.* (*M.*) *nigerrimus*. It might be argued that such an effect might be due to the increasing difficulty of catching as daylight fades and is replaced by artificial light. However, experience of the method does not support this view and in any case an effect of this sort would be expected to affect all the species at the same time in relation to sunset ; there are considerable differences in the time of peak activity in the several species ; it fell in catch period 8 in the case of *C. centurionis*, in period 11, 30 minutes later, in the case of *T.* (*M.*) *africanus*. The peak period of activity is usually clearly defined and it is an episode which for any given species is subject to only slight movement in time in relation to sunset. In the analyses of the data by individual platforms, by catch groups and by types of weather condition it was the exception for the time of the peak activity to be shifted more than one catch period away from the position which it occupied over the whole series. Of the 23 individual evenings on which clearly defined curves for *A.* (*M.*) *gambiae* could be derived, 21 show the peak either in the same catch period as does the whole series or in that immediately before or after. Practically the whole range of variation is thus included within three successive ten-minute periods, *i.e.*, between the mid-points of the first and third, within 20 minutes. The same range of variation includes all the five curves for individual evenings for *T.* (*M.*) *africanus*, eight out of eleven of those of *A.* (*S.*) *africanus*, and 15 out of 20 of those of *C. centurionis*. No definite results attended attempts to correlate what variations took place in the time of peak activity with the information available on climatic conditions and on the characteristics of the various platforms. However, the remarkable regularity of occurrence of the peak period of biting activity indicates that it is much more likely to be related to light than to temperature or humidity conditions which latter are subject to wider variation from day to day. Some confirmation of this hypothesis was obtained from the data relating to *A.* (*M.*) *gambiae* in which there was an indication that an early peak was more likely on a cloudy evening. Further, the onset of the main activity in *A.* (*M.*) *gambiae*, *A.* (*S.*) *africanus* and *C. centurionis* tended to be later on moonlit than on moonless nights. Mellanby (1940) has shown that light of an intensity of 6 metre-candles almost entirely prevented the appearance of *Blatta orientalis* L. in an animal house and even lower intensities greatly reduced the numbers appearing. The crepuscular behaviour of the forest insects studied suggests that a similar mechanism affects them, the inhibitory effect of light being removed as daylight fades. It follows that the changes in microclimatic conditions in the resting places of the insects are more likely to be of importance than those on the catching platforms themselves. More precise studies will be required fully to confirm this postulate but the present work has shown that the curve of activity near sunset is sufficiently characteristic to provide a marker suitable for studies of this kind. The use of the peak period of activity as a marker has certain disadvantages in that it is likely to be a result of several factors including at least the change of conditions in the resting places, leading to the release of the insects, and the distances, both vertical and horizontal, between the main resting places and the place of the catch. The time of the onset of the main activity would perhaps be preferable but it is a point less easily susceptible of definition than is the time of peak activity, particularly in those species, *e.g.*, *A.* (*S.*) *africanus*, which maintain a low level of activity during the daylight hours.

Haddow (1945), Haddow & others (1947), and Mattingly (1949a, b) have studied the biting rhythm of blood-sucking insects with particular reference to the forest environment. They are agreed that certain distinct types of biting cycle can be distinguished and their curves, which are related to Local Mean Time and cover all or most of the 24-hour period, offer numerous examples. As far as species mainly active by night are concerned, the following types are recognised :—

Type of *A.* (*S.*) *africanus*.—A single peak of biting activity occurs, in the 18–19 hours period ; activity is fairly high in the hour preceding this period and in the two succeeding hours but never exceeds low levels at other times of day (Haddow & others, 1947). Mattingly's curve (1949a) is closely similar, differing only in that he found a slightly higher relative activity in the later night hours.

Type of *A.* (*M.*) *gambiae*.—Activity, which is at a low level during the daylight hours, increases about sunset ; this trend is continued fairly steadily through the night, and activity reaches a peak in the 05–06 hours period (Haddow & others, 1947).

Type of *T.* (*M.*) *africanus*.—Activity, which is at a low level during the daylight hours, increases about sunset, rises to a maximum about the middle of the night and falls away again before dawn ; the position in time of the maximum varied in different catches between the hours ending 21.15 and 05.15 hours (Mattingly, 1949a). Haddow & others (1947) found high activity throughout the night but the most in the four hours following sunset.

Several factors have combined in this previous work to obscure the short-term variations in biting activity which take place near to the time of sunset. It was inevitable in pioneer studies that time divisions of considerable duration (one hour) were used as it was required first to define the general form of the curve through the 24 hours. In many cases, when numbers were small, it was necessary to combine several successive hours into groups. The yearly movement of the time of sunset was not taken into account so that when a large change of activity took place near sunset, part at least of that change might fall into one or other of two successive hours (L.M.T.) depending on the time of year at which the catch was performed. However, Mattingly (1949a) suspected the presence of a short-term peak of activity shortly after sunset in *A.* (*M.*) *gambiae* in his own work and drew attention to a probable similar effect in Haddow's data. Mattingly considered that it was likely that *A.* (*M.*) *gambiae* would be found to show two peak periods of activity, one in the evening and one in the morning, both at about the same light intensity, " during the period of Nautical Twilight ", *i.e.*, between the times of Civil and Nautical Twilight. The present work has confirmed Mattingly's belief that *A.* (*M.*) *gambiae* shows a short-term peak of activity shortly after sunset and has, further, shown that a similar occurrence takes place in all the species whose numbers are adequate for study. This finding suggests that the three types of curve described above for nocturnally-active species are only perhaps different degrees of the same phenomenon, and that all may be explained in the following manner :—

(*a*) During the daylight hours, light constitutes an inhibiting factor which largely or entirely limits the activity of insects of a nocturnal habit. During this enforced inactivity there is an accumulation of hungry individuals in the population.

(*b*) When the influence of daylight is removed by sunset, the insects are released into activity and those individuals which are immediately ready to feed, and which have been resting in the forest close to the catching stations, pass to the bait and are the main cause of the peaks of biting activity observed shortly after sunset. The different times of peak activity in different species may be an expression either of species differences in the critical level of light intensity allowing activity or of factors affecting the length of time taken by a species to pass from the resting places to the experimental platforms.

(*c*) Those individuals biting later in the night are those which were not ready to feed immediately after their release by sunset from the inhibitory effect of daylight and which only entered the condition of readiness to feed at a later hour.

(*d*) The different types of curve are due to differing proportions of these two population groups in the populations of different species of insect. The respective constitutions of these two different population groups are at present a matter for speculation only, but in mosquitos, and probably also in *Chrysops*, one blood meal, the first, differs from all others in that it is preceded by mating. The question may be discussed with this as a basis.

Evidence is produced from the present work that individuals of *C. centurionis* feeding for the first time do so by daylight. It is thus highly probable that those specimens feeding by night are mainly those feeding for a second or subsequent occasion. The biting-activity curve produced by this species is very clearly of the *A.* (*S.*) *africanus* type, indeed its similarity to the curve of that species has been especially emphasised by Haddow & others (1950) who found that 115 specimens of a total of 141 occurred in the hours 17–22 and only 16 in the rest of the night. It may be tentatively deduced then that the initial peak shown by *A.* (*S.*) *africanus*, and by the other species also, is due mainly to individuals feeding for the second or subsequent occasion and that these compose the group of insects ready to feed immediately on their release into activity by the fading of daylight. The corollary is that those individuals which come to the bait later in the night, those which were not immediately ready to feed on their release into activity, are the newly-hatched individuals feeding for the first time. Little is known of the swarming and mating habits of forest mosquitos but most of the descriptions of the mosquito swarming deal with crepuscular species under twilight conditions (Bates, 1949) and it seems likely that the mating of newly-hatched females would be a factor postponing their coming for a blood meal to some time later than their first release into activity by the fading of daylight. On these postulates, supposing further that the proportions in which these two categories (individuals feeding for the first, and for the second and subsequent occasions) are represented in the total population differ in different species, all the three typical nocturnal rhythms may be explained. If the nightly addition of individuals feeding for the first time is small in proportion to the total population then a curve of the type of that of *A.* (*S.*) *africanus* will be produced. If it is large, the addition of individuals ready to feed, into the population later in the night will provide a curve of the type of those of *A.* (*M.*) *gambiae* or *T.* (*M.*) *africanus*. These two latter curves may be considered to differ only in the time at which the nightly addition of new individuals begins to fail ; in *A.* (*M.*) *gambiae* it may be considered to last until the advent of daylight at dawn cuts short activity, in *T.* (*M.*) *africanus* to fail about the middle of the night.

The hypothesis may be compared with other evidence. Of the 14 curves for different species of mosquito given by Mattingly (1949a, b), 11 are of insects mainly nocturnally active which give curves of one or other of the types described above. Five (*Aëdomyia africana*, *Aëdes* (*S.*) *africanus*, *A.* (*D.*) *taylori* Edw., *A.* (*D.*) *flavicollis* Edw. and *Culex* (*C.*) *thalassius* Theo.) yielded curves of, or approaching to, the *A.* (*S.*) *africanus* type. The other six showed biting rhythms of the type of *Anopheles* (*M.*) *gambiae* or *T.* (*M.*) *africanus* and were *A.* (*A.*) *paludis* Theo., *A.* (*M.*) *moucheti* Ev., *hargreavesi* Ev., *gambiae*,* *T.* (*M.*) *africanus* and *Aëdes* (*M.*) *grahami*. Of the first five species which yield curves of the *A.* (*S.*) *africanus* type, three breed typically in tree holes (*A.* (*S.*) *africanus*, *A.* (*D.*) *taylori* and *flavicollis*) ; *C.* (*C.*) *thalassius* breeds mainly in small bodies of water, and larvae of *Aëdomyia africana* are typically associated with *Pistia* (Hopkins, 1936). At least four of these species, therefore, breed in bodies of water of a circumscribed character and it seems likely that they can rarely

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*Mattingly states his belief that the *A.* (*M.*) *gambiae* here referred to contained a high proportion of *A.* (*M.*) *melas* Theo.

produce really large adult populations ; this is borne out by Mattingly's catches in which the numbers of these species taken in twenty-two 24-hour catches were, respectively, 60, 525, 384, 695 and 161. Of the second six species, which yielded curves of the *A.* (*M.*) *gambiae* or *T.* (*M.*) *africanus* types, all breed typically in bodies of water of considerable size (Edwards, 1941, pp. 467 *et seqq.*) and their larval populations, and hence their capabilities to produce large adult populations, are not liable to be limited by the extent of the available breeding place ; this is again largely borne out by the numbers taken by Mattingly in the same series of catches which were, respectively, 301, 151, 6,700, 2,502, 18,175 and 1,083. It seems, then, that those species whose breeding places are circumscribed and whose adult populations are probably never really large, tend to yield biting activity curves of the *A.* (*S.*) *africanus* type and those whose breeding places are extensive and which may produce very large adult populations tend to yield curves of the *A.* (*M.*) *gambiae* or *T.* (*M.*) *africanus* types. In a species whose breeding places are extensive, and which hence may produce large adult populations, it would seem less essential that individual adults should be long-lived, passing through many successive ovarian cycles. In those whose habits are to breed in circumscribed and transitory breeding places such a habit would seem to be of high value. The former group will thus be expected to contain a larger proportion of young females in its population than will the latter, and the biting-activity curves will be expected to approximate to the *A.* (*M.*) *gambiae*—*T.* (*M.*) *africanus* types and to the *A.* (*S.*) *africanus* type, respectively.

Numbers biting hour by hour through the 24 hours are given by Haddow & others (1947) for *A.* (*M.*) *gambiae* and by Mattingly (1949a) for *A.* (*M.*) *gambiae* and *T.* (*M.*) *africanus*. The period of initial activity about sunset* for these species is defined by the present work and is the three hours from one hour before to two hours after sunset (fig. 4). The numbers of mosquitos occurring in the period of initial activity may be compared with those occurring in the rest of the 24 hours in the figures given by these workers. The figures are as follows :—

	A. (*M.*) *gambiae*		*T.* (*M.*) *africanus*
Initial period of activity	2,320	178	2,218
Rest of the 24 hours	27,920	2,324	15,957
Percentage	8·3 (Haddow)	7·7 (Mattingly)	13·9 (Mattingly)

The percentage given is the proportion which the catch in the initial period of activity bears to the catch in the rest of the 24 hours. On the hypothesis described above this percentage will represent the number surviving to the second and subsequent blood meals out of an initial population of 100. It may well be too low as all the specimens biting by daylight have been included in the postulated initial population and some of them may be feeding for the second or subsequent occasion. However, even the rate of reduction indicated does not seem unlikely. The most precise information available on mosquito longevity is in the work of Russell and Ramachandra Rao (1942) who studied the survival of laboratory-bred *Anopheles* (*M.*) *culicifacies* Giles in an open-air insectary which simulated as closely as possible natural conditions. They concluded that the death rate of adult females approximated to 50 per cent. every two days. Their actual results show an even more rapid reduction than this in the initial few days ; all the plotted values on their figure (Chart 2) are on the left of the geometric curve until the tenth day after hatching. On the four occasions on which the population was reduced to between 12 and 13 per cent., this reduction took place in six, seven, five and four days respectively. In an experiment of this sort it is probable that the mosquitos are at least to some extent protected from their normal predators and even higher death rates are to be expected in nature. The time interval between successive blood meals is unknown in any forest insect but it is estimated to be 48 hours for *A.* (*M.*) *gambiae* in native huts in Bwamba (Lumsden, 1951b) ; it will almost certainly be longer in the cooler environment of forest.

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*This period has been referred to earlier, when the crepuscular period only was being described, as the period of main activity.

Haddow (1945) and Haddow & others (1947) have conducted detailed examinations of the forest microclimate, comparing it with that of other environments and also the microclimates of the various levels within it, and have attempted to correlate climatic changes with changes in the biting activity of various insects. In general, it is felt that such comparisons have yielded disappointing results at least with the species which are nocturnally active. For instance, he (1945) suggested that the biting activity of *A.* (*M.*) *gambiae* is mainly influenced by temperature, or saturation deficiency, or both, the relation being an inverse one, activity increasing through the night while the temperature and saturation deficiency are falling ; light was considered as not likely to be the most important factor as the catch was quite large in the period 06 to 10 hours, in full daylight. The climatic conditions through the night in forest of this type are, however, remarkably equable ; the relevant tables of Haddow (1945) show that between 19 and 05 hours the seven-day mean values of temperature decline 3°C. from about 22°C., those of relative humidity rise 2 per cent. from 88 per cent., and those of saturation deficiency decline about 1 mm. from 2·5 mm. It is not impossible that such small differences have an effect as Thomson (1938) has shown such degrees of discrimination in the case of *Culex fatigans* Wied. in laboratory experiments. However, it should be remembered that his experiments related to the choice of a resting place only ; in relation to the taking of a blood meal it is difficult to understand his finding that hungry females showed avoidance of the higher of two temperatures as low as 15°C. Lumsden (1947) found, in laboratory experiments with hungry *A.* (*S.*) *aegypti* (L.), that the optimum environmental temperature for biting was near 35°C. and that the relative humidity at the place and time of biting appeared to be of little or no importance. He found that the proportion of biting individuals increased from lower temperatures up to the optimum at about 35°C. ; a similar effect would have been expected on the biting activity of *A.* (*M.*) *gambiae* in forest so long as the temperatures did not approach lethal heights, rather than the reverse relationship postulated by Haddow. Nor does it seem easy to account for the middle night peak activity in the case of *T.* (*M.*) *africanus* by microclimatic influences which are subject to such small and gradual changes through the night.

The *experimentum crucis* to test the validity of the hypothesis now advanced has not yet been performed. It is the estimation of the age of the mosquitos coming to feed period by period through the night by means of ovarian examinations. Valuable information might also be obtained from the dissection of the salivary glands of mosquitos in an area where the malaria sporozoite index is normally high ; it is indeed remarkable that this has not already been studied, as it bears an obvious relationship to the problem of preventing infection, but no record of such a study has been found in the literature. It may well be that the most dangerous time for infection with malaria is not, as Mattingly (1949a) suggests, in the later part of the night when biting activity is at a maximum, if at that time it is confirmed that most of the mosquitos are feeding for the first time.

Summary.

The results of a series of catches of biting insects at human bait on platforms in the canopy of the forest at Mongiro, Bwamba, Uganda, are reported. The catches were designed to investigate differences in the numbers taken, due to platforms,

season and weather, but mainly to study the short-term changes in the activity of biting insects near the time of sunset. Each catch lasted from 3 hours before to 3 hours after sunset, and was divided into 10-minute periods from 30 minutes before to 1 hour after sunset. The main analysis is of catches made on five platforms on each of 100 evenings which covered the end of the dry and beginning and end of the wet season, 1948, and the end of the dry season, 1949.

A list of the species of biting insects encountered is given. Eight species (*Anopheles* (*M.*) *gambiae*, *Taeniorhynchus* (*C.*) *fuscopennatus*, *Taeniorhynchus* (*M.*) *africanus* and *uniformis*, *Aëdes* (*M.*) *nigerrimus*, *Aëdes* (*S.*) *apicoargenteus* and *africanus*, and *Chrysops centurionis*) were found to be abundant and data relating to them has been subject to more or less detailed analysis. The remaining 27 species or groups of species are discussed shortly.

Six of the eight abundant species show statistically significant differences between the catches on the five separate platforms. Arrangement of the five platforms in order of the total numbers taken, for each of these six species, gives results which are practically identical in three species (*Anopheles* (*M.*) *gambiae*, *T.* (*M.*) *africanus* and *uniformis*) and in two others (*Aëdes* (*S.*) *apicoargenteus* and *africanus*) ; however, the arrangements in these two species groups differ widely from each other. The arrangement for *C. centurionis* differs again.

These ordinal arrangements are compared with the obvious characteristics of the platforms and no consistent relationship is found. It is pointed out that the first three species are derived, largely or even entirely, from breeding places outside the forest, in or associated with the adjacent extensive areas of open swamp, while the last three are likely to be completely confined to the forest for breeding ; both the latter two mosquito species breed in tree holes, and dense shade is characteristic of the breeding places of species of *Chrysops* closely allied to *C. centurionis*. It is considered most probable that these platform differences in the numbers yielded are due to local differences in the densities of the adult populations which in turn are related to the abundance and proximity of breeding places.

Seasonal variations in the numbers of the abundant species are also studied and are considered to be almost certainly related to the amount and distribution of rainfall affecting the profusion of breeding places at least as far as the mosquito species are concerned. The tree-hole breeding species, however, suffered a reduction in numbers before the end of the wet season, an effect also noted by Mattingly in West Africa.

The amount of the reduction of the numbers of *A.* (*S.*) *africanus* in the dry season is of importance in assessing the possibilities of the survival of yellow fever virus in that mosquito as a host. The estimated reduction in the dry season of 1949 was to less than one twenty-third of the population existing at the end of the previous wet season (November, 1948).

C. centurionis occurred during all the catch series but was extraordinarily abundant in late March and early April, 1948. It is considered probable that this time of increase represents a yearly period into which hatching from the pupa is compressed. This conclusion is supported by the finding that during this period the biting population was composed of two groups one of which tended to bite before, and the other after, sunset.

Evidence is produced to show that the biting activity of *Anopheles* (*M.*) *gambiae* was depressed by wind, that of *Aëdes* (*S.*) *africanus* by wind and rain but that that of *C. centurionis* was little affected by either of these factors.

Studies of the biting-activity changes in the nine ten-minute periods extending from 30 minutes before to one hour after sunset are presented. Each of the species which were known to be mainly active by night and which were sufficiently abundant for study, show activity increasing about sunset, rising to a maximum shortly after, and thereafter declining again to moderate levels. This initial peak is very clearly marked in the cases of *Anopheles* (*M.*) *gambiae*, *T.* (*C.*) *fuscopennatus*, *T.* (*M.*) *africanus*, *Aëdes* (*S.*) *africanus* and *C. centurionis*, less so, but still distinct, in *T.* (*M.*) *uniformis* and *A.* (*M.*) *nigerrimus*. Its position differs in different species, being earliest in the case of *C. centurionis* (18.10–18.20 hours) and latest in that of *T.* (*M.*) *africanus* (18.40–18.50 hours). However, in any given species the peak period of biting activity was subject to only slight movement in time in relation to sunset ; in analyses of the data by different platforms, by season, and by types of weather it was the exception to find the time of peak activity to be shifted more than one ten-minute period away from the position which it occupied over the whole series. Further, in studies of the curves of biting activity on individual nights, three consecutive ten-minute periods centred on the period which showed the peak over the whole series, included the peak in 21 of the 23 *A.* (*M.*) *gambiae* cases available, in all five of the *T.* (*M.*) *africanus*, in eight of the 11 *A.* (*S.*) *africanus* and in 15 of the 20 *C. centurionis*, cases. The regularity of the occurrence of the peak period of activity in any given species is considered to indicate that it is largely controlled by changes in light intensity about the time of sunset.

These findings are discussed in relation to the general problem of the mechanism controlling the rhythm of activity in insects biting by night and a hypothesis, which is based on the conception that different population groups may have different biting habits, is advanced to account for the different types of cycle commonly encountered.

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DDT RESISTANCE IN AN ITALIAN STRAIN OF *MUSCA DOMESTICA* L.

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Although DDT-resistant house-flies (*Musca domestica* L.) were first reported by Saccà in 1947, and papers on the subject have appeared from Italy, Switzerland, Denmark and America, little light has been thrown on some sides of the problem. Indeed many published findings appear contradictory. Different fly stocks show very different degrees of resistance. In some cases resistance is scarcely discernible ; in others it is stated to be as much as 500-fold. DDT resistance is reported to have declined in some strains that have been cultured without coming into further contact with DDT, but other strains kept under similar conditions have maintained resistance. In the field, insecticides such as gamma BHC and chlordan have controlled DDT-resistant flies, but laboratory strains have been bred resistant both to these insecticides and to a variety of chemically unrelated insecticides. It is clear that one can produce resistant strains in the laboratory by selection, but one cannot say with certainty that resistance can be produced in this way in any population.

This paper deals with two strains of house-flies, one (Roma) non-resistant, and the other (Torre in Pietra) DDT-resistant, which were obtained in the spring of 1948 from Professor Missiroli of the Instituto Superiore di Sanità, Rome. These strains were tested for resistance when first acquired and again after a long period of laboratory culturing. Attempts were made

(*a*) to increase the resistance of Torre in Pietra strain ;

(*b*) to derive strains resistant to DDT and pyrethrins from the Roma strain ;

(*c*) to determine whether these selected strains had any increased resistance to other insecticides.

Methods.

Breeding technique.—Flies were bred in a constant temperature room at 26° to 27°C. with a relative humidity between 45 and 60 per cent. The breeding medium was the Ministry of Health standard guineapig meal mixed with water in the proportion of 50 gm. of meal to 50 cc. of water. Eggs were placed on the medium in $2\frac{1}{2}$-lb. jars giving approximately 1,500 eggs per kg. of medium (wet weight). Puparia separated from the medium by floating in water, were transferred to 6-in. cube cages, and on emergence flies were fed with sugar and a solution of 50 per cent. milk and 50 per cent. water.

Standardisation of flies.—It is important that insects of different strains or batches used in comparative insecticidal tests should be bred under the same conditions and should be standard in all respects. Preliminary tests in which flies of the Roma strain were exposed to filter papers impregnated with DDT gave the following information :—

(1) Male flies were more susceptible to DDT than female flies.

(2) Flies 9–10 and 16–19 days old were more susceptible to DDT than 4–5-day-old flies. Differences between flies of the 9–10- and 16–19-day-old age groups were not so distinct.

(3) Small flies were more susceptible to DDT than large flies of the same strain.

Clearly then, flies used in comparative toxicity tests should be of standard sex, age, and size. In further experiments flies of 4–5 days old only were used, and counts made to determine the sex ratios so that figures for the percentage kill could be corrected accordingly. Size was kept constant as far as possible, but this is difficult to control since it depends on conditions in the culture medium.

Testing technique.

(*a*) Preparation of test surfaces. The three types of test surface used were Essex board*, filter papers, and glass cylinders.

Six-in. squares of Essex board were sprayed with solutions of DDT or gamma BHC by means of the spinning disc sprayer which distributes the insecticide evenly over the surface (Parr & Busvine, 1948). The DDT was pure p,p′DDT (m.p. 108·4–108·7°C.) and the solvent odourless distillate. The gamma BHC was prepared from liquid concentrate L.G.140 (this contains 10 per cent. gamma BHC w/w) diluted to the necessary concentration with odourless distillate. The Essex boards were left to dry overnight before use.

Filter papers were impregnated, by the method described by Busvine and Barnes (1947) with DDT dissolved in acetone, gamma BHC in acetone, or with pyrethrins diluted with ether from a 20 per cent. concentrate. After drying overnight the filter papers were used to line the tops and bottoms of petri dishes in which the insects were exposed.

Glass cylinders and petri dishes were treated with solutions of DDT in acetone, or pyrethrins in ether (see Busvine, 1949) giving a deposit of 10 mg. per sq. ft. of DDT and a deposit of 0·1 mg. per sq. ft. of pyrethrins.

(*b*) Exposure of flies. The tests were carried out in a constant temperature room at 26–27°C. Samples of flies collected from the cages in test tubes were anaesthetised lightly with carbon dioxide and then exposed to the treated surface. Flies recover rapidly from the small dose of carbon dioxide given as an anaesthetic. Laboratory experiments in which house-flies were exposed to a saturated atmosphere of carbon dioxide for several hours show that a considerable dose is necessary to prove harmful.

In tests with Essex boards treated with DDT, flies were confined between the two treated surfaces by a narrow celluloid ring. Unlike DDT, gamma BHC has an insecticidal vapour and since it was intended to investigate the contact rather than the fumigant effect of this insecticide, perforated zinc rings were used for separating the treated surfaces. After exposure for 1 hour the insects were transferred to clean cages and the percentage mortality determined after 24 hours. A similar technique of exposure was used in tests with treated filter papers.

In cylinder tests flies were exposed to the treated cylinders inverted over treated petri dishes, and the number of flies which had fallen on to their backs was recorded at intervals of 2½ or 5 minutes.

Criterion of resistance.—Either the time of exposure to a treated surface can be kept constant and the equitoxic doses determined for each strain of flies, or one concentration of insecticide used and the time required to give the same " knock-down " in each strain determined. Comparison of the two strains of flies was made on the basis of the lethal dose 50 (the concentration of insecticide required to give a 50 per cent. kill) or the lethal time 50 (the time taken to give 50 per cent. knockdown of flies) which was estimated visually from the log.-probit regression lines in some cases, or calculated from the data as described by Bliss (1935).

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*Essex board is a compressed cardboard, ½ cm. thick.

Results.

The resistance of the two Italian strains of house-flies to DDT.

The resistance of the Roma, and Torre in Pietra strains was determined shortly after they were obtained in 1948. Table I gives the percentage kill of flies in these two strains when exposed to eight concentrations of DDT on Essex board.

TABLE I.

Kill of flies of the two strains when exposed to residual deposits of DDT on Essex board.

Concentration of DDT mg. sq. ft.	Percentage kill after one hour exposure	
	Strain Roma	Strain Torre in Pietra
300	97	49
183	100	70
170	97	55
88	82	35
87	68	23
49	75	19
40	27	9
20	2	5
Average number flies per concentration ...	32	34

The inconsistencies in the results may be due to the fact that the tests at all concentrations were not carried out on the same day or the same generation of flies: each pair of results, however, is comparable and in each there is a distinctly higher kill in the Roma strain.

The calculated probit-log concentration regression lines were :—

strain Roma ... $Y=3{\cdot}3351x-0{\cdot}7627$
$\chi^2=5{\cdot}468$
$n=6$

strain Torre in Pietra $Y=1{\cdot}6476x+1{\cdot}2443$
$\chi^2=10{\cdot}0743$
$n=6$

The χ^2 which is a measure of the departure of the actual observations from the fitted probit-log concentration line does not exceed the expected value for $P=0{\cdot}5$ in either strain. The populations are therefore homogeneous and the difference between the two strains a real one.

The L.D.50 was 53·4 mg. sq. ft. for the Roma strain and 190·3 mg. sq. ft. for the Torre in Pietra strain.

The Torre in Pietra strain could therefore withstand a concentration of DDT 3·6 times greater than the Roma strain.

The resistance of the Torre in Pietra strain declined when bred in the laboratory for a number of generations without coming into contact with DDT. The resistance is compared in Table II on the basis of the median lethal dose which has been calculated from the data in each case.

Fig. 1 shows that the probit-log concentration lines are not parallel.

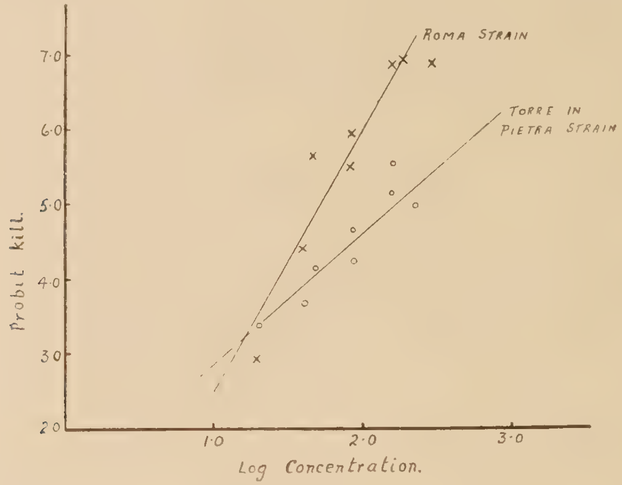

Fig. 1.

TABLE II.

The decline in resistance to DDT of the Torre in Pietra strain when bred in the laboratory without coming into contact with DDT.

Date	Generation	L.D. 50 in mg./sq. ft.		Ratio R/Tp
		Strain Roma (R)	Strain Torre in Pietra (Tp)	
May–June 1948	4–5	53·4	190·3	1 : 3·56
Nov. 1948	14	53·7	68·8	1 : 1·28
June 1949	22	61·7	68·7	1 : 1·11
Nov. 1949	27	64·2	80·2	1 : 1·25

The resistance of the two Italian strains of house-flies to gamma BHC and pyrethrins.

The results with gamma BHC were very variable (fig. 2) but on the basis of the L.D.50 the resistance of the two strains seems to be almost identical. The calculated L.D.50 for the Roma strain was 2·8 mg. sq. ft. and for the Torre in Pietra strain 3·1 mg. sq. ft.

The difference between these two figures is clearly not significant. The variability is probably due to the fact that the tests were not all carried out on the same day.

The percentage kill of house-flies when exposed for 1 hour to filter papers impregnated with pyrethrins is given in Table III.

The DDT-resistant strain is more resistant to pyrethrins than the non-resistant strain.

Selection experiments. Selection experiments on the Torre in Pietra strain were started in the spring of 1949 when the resistance to DDT had become slight. Flies surviving from 1-hour exposures to filter papers impregnated with 1·0 mg./sq. cm. of DDT were allowed to breed, and this process of selection was repeated over eight generations. Although there is a fluctuation in the percentage kill obtained from generation to generation there is clearly an increase in resistance to DDT over this period of selection (see Table IV).

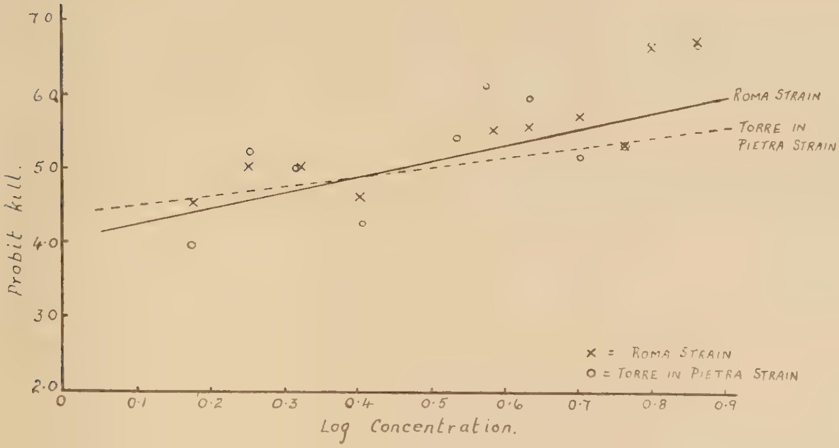

Fig. 2.

TABLE III.

Kill of flies of the two strains exposed to filter papers impregnated with pyrethrins.

Concentration of pyrethrins mg./sq. cm.	Percentage kill of flies after exposure for 1 hour	
	Strain Roma	Strain Torre in Pietra
1·0	98	64
0·1	92	31
0·01	17	1

Since these figures refer only to the percentage kill obtained with one concentration of DDT it is impossible to say by how many fold the resistance was increased. A cylinder test in the ninth generation indicated a clear difference in resistance

TABLE IV.

Kill of flies of the Torre in Pietra strain over eight generations of selection with DDT.

Generation of selection	Percentage kill when exposed for 1 hour to filter papers impregnated with DDT at the rate of 1·0 mg./sq. cm.	
	Strain Torre in Pietra	Strain selected from the Torre in Pietra strain (X)
1	83	64
2	76	21
3	93	47
4	94	43
5	86	11
6	47	5
7	—	—
8	92	0

between the selected and Torre in Pietra strains. The lethal times 50 were : Torre in Pietra strain, 12·4 minutes ; and as far as could be ascertained for the selected stock 100 minutes. In fact it is not possible to fit probit-log concentration regression lines for the selected stock because the knock down curve is not symmetrical. Fig. 3 shows that the curve flattens abruptly between the 40 and 60 per cent. knockdown levels.

Tests on the 22nd generation of the selected strain (X) after it had been cultured without contact with DDT for 12 generations show that there is little decline in resistance in this strain (see fig. 3).

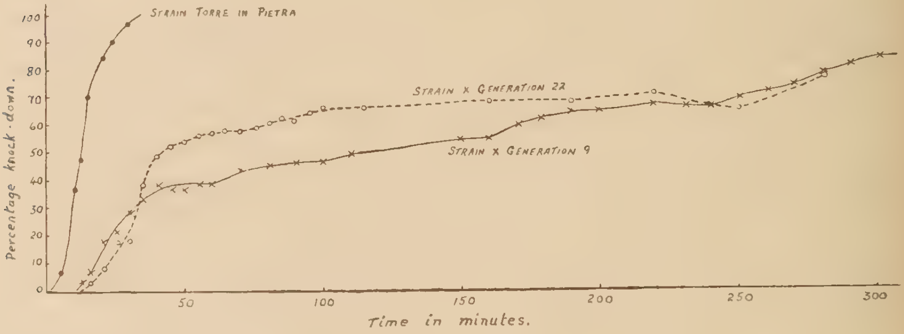

Fig. 3.

The increase in resistance to DDT observed after selection on the non-resistant Roma strain of flies was negligible. Flies were exposed in DDT-treated glass cylinders until approximately 50 per cent. were knocked down, and the survivors were transferred to clean cages and allowed to breed. In each generation the selected stock was compared in a knockdown test with flies of the Roma strain. In Table V the two stocks are compared on the basis of the median lethal time which was estimated visually from the probit-log time graphs.

TABLE V.

Knockdown of flies of the Roma strain, and of a stock derived from it by selection with DDT.

Generation	Median knockdown time in minutes		Ratio R/Y
	Strain Roma (R)	Selected strain (Y)	
1	17·78	19·95	1 : 1·12
2	12·59	13·80	1 : 1·10
3	14·45	19·05	1 : 1·32
4	22·91	19·95	1 : 0·87
5	17·38	17·38	1 : 1·00
6	13·80	17·38	1 : 1·26
7	19·95	19·95	1 : 1·00
8	16·60	23·99	1 : 1·44

Selection on the Roma strain with pyrethrins, however, gave a slight increase in resistance to this insecticide. Table VI gives the increase in resistance to pyrethrins after selection over eight generations with this insecticide.

TABLE VI.

Knockdown of flies of the Roma strain, and of a stock derived from it by selection with pyrethrins.

Generation	Percentage survivors to give next generation	Median knockdown time in minutes		Ratio R/P
		Strain Roma (R)	Selected strain (P)	
1	26	5·2	6·9	1 : 1·33
2	7	5·2	6·9	1 : 1·33
3	14	7·2	10·9	1 : 1·51
4	42	6·6	17·4	1 : 2·64
5	40	7·2	20·0	1 : 2·77
6	20	10·0	22·13	1 : 2·21
7	30	13·8	54·95	1 : 3·98
8	44	16·6	31·62	1 : 1·90

Tests with gamma BHC, DDT, and chlordan, demonstrated that this resistance to pyrethrins was not specific. The median knockdown times with DDT were 15 minutes for the Roma strain and 21 minutes for the pyrethrin-selected strain. Similarly, tests with filter papers treated with gamma BHC and chlordan gave a slightly lower kill at each dosage in the pyrethrin-selected stock than in the Roma strain.

Discussion.

When first received, the flies from Torre in Pietra were definitely more resistant than those from Rome, although the degree of resistance was small compared with the Swedish strain described by Wiesmann (1947) and the strains recently reported from America (March & Metcalf, 1949). Apart from the greater resistance shown in insecticidal tests, there was a difference in the dosage mortality curve which was flatter for the Torre in Pietra than for the Roma strain. In other words, the population of the Roma strain had a smaller range of resistance, and was more homogeneous ; whereas the Torre in Pietra strain contained susceptible as well as moderately resistant individuals. These differences perhaps explain the success or failure of selection experiments. In the Roma strain selection for DDT resistance failed, yet similar selection in the Torre in Pietra strain gave a rapid increase in resistance. Since the Roma strain appears to be comparatively pure for non-resistance this failure of selection is not surprising. There seems also to be a correlation between a high degree of DDT resistance (X strain) and the ability to maintain resistance after exposure to DDT has ceased. This would be expected if the resistant strain was also nearly pure.

The resistance of DDT-resistant strains of house-flies to other insecticides admits of no simple explanation. The Torre in Pietra and X strains were not resistant to γ BHC but had a slight resistance to pyrethrins. Results from America (Barber & Schmitt, 1949), Denmark (Keiding & van Deurs, 1949) and Italy (Mosna, 1949), indicate that resistance to DDT and its analogues is fairly specific. On the other hand, there are reports (Wilson & Gahan, 1948 ; March & Metcalf, 1949) of strains with a marked DDT resistance having a slight resistance to a number of insecticides. A line selected for pyrethrin resistance from the Roma strain had a similar general resistance which may have been due to an increase in general vigour.

Summary.

1. Flies of 9–10 and 16–19 days old were more susceptible to DDT than 4–5-day-old flies. Female flies were less susceptible than males, and large flies less susceptible

than small flies to residual deposits of DDT. It is clearly important that flies used in comparative toxicity tests should be standardised for age, sex and size.

2. The strain Torre in Pietra was 3·6 times more resistant to dry deposits of DDT on Essex board than the Roma strain, but this resistance declined after culturing the strain in the laboratory for 22 generations.

3. The DDT-resistant strain (Torre in Pietra) was as susceptible as the non-resistant (Roma) strain to deposits of γ BHC on Essex board but was slightly resistant to pyrethrins.

4. The DDT resistance of the Torre in Pietra strain was increased readily by selection, yet a similar selection with DDT on the Roma strain was unsuccessful. This failure in the Roma strain may be due to the purity of the stock.

Acknowledgements.

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STUDIES ON AQUEOUS SUSPENSIONS OF INSECTICIDES. PART II. QUANTITATIVE DETERMINATIONS OF WEIGHTS OF DDT PICKED UP AND RETAINED.

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A relationship between the size of DDT particles and their effectiveness against mosquitos, *Aëdes aegypti* (L.), exposed to them for short contact periods has been established in earlier experiments (Hadaway & Barlow, 1951).

Weights of DDT picked up and retained by mosquitos and tsetse flies have now been determined chemically and correlated with their toxic effects. Results given in this paper confirm and amplify previous observations.

Materials and Methods.

Techniques for the preparation and spraying of aqueous suspensions of DDT crystals and for the exposure of mosquitos to the deposits for short contact periods have been described previously (Hadaway & Barlow, 1951). Tsetse flies were exposed individually.

The amount of pp′DDT picked up by the insects was measured by a method based on that of Schechter and others (1945), the variations used here being in the extraction procedure after nitration.

After some preliminary investigations, the operations were standardised as described below. One important cause of error was loss of DDT if the heating was continued after the last trace of extraction solvent had been removed (see graph, fig. 1). A small quantity of stearic acid, about 1 mg., was added to each sample to prevent this loss (see Prickett & others, 1950). This material, however, gave a small blank colour in the final sodium methoxide-methanol solution when the procedure for extraction of the nitration product as described here was followed.

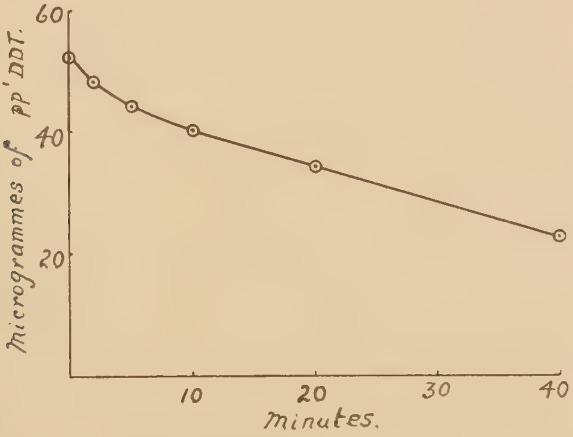

Fig. 1.—Loss of pp′DDT on heating.

Its use was therefore discontinued. Instead, the heating of each sample was continued for exactly one minute after the last visible trace of solvent had evaporated.

In this time, all the benzene was removed and therefore could not interfere by yielding nitrated products, while the loss of DDT was actually less than the error of the method. This routine for evaporation of the solvent was followed for preparation of the calibration curve and for analysis of washings from insects. It was repeatable and gave results of sufficient accuracy for our present purpose.

Preparation of sample.

For determinations of initial pick-up, each batch of insects was removed as usual from the exposure funnel with the aid of carbon dioxide anaesthesia, placed in a 2×1-in. specimen tube and killed with chloroform vapour. In retention tests, the insects were removed from recovery cages in the same way.

The insecticide on the insects was removed by washing with benzene. For this purpose, the insects were transferred as quickly as possible to a 50-ml. conical flask and washed four times with 5 ml. benzene each time. The benzene used had been distilled and the first and last fifths discarded. The extract was filtered through a cotton wool plug, which had been washed with benzene, into a 100-ml. conical flask and most of the solvent removed on the water bath with the aid of a stream of air. The remaining solution was now transferred to a $6\times\frac{3}{4}$-in. test tube and the remainder of the benzene removed on a water bath with an air stream, observing the time interval mentioned above.

Estimation of DDT.

The residue in the tube was treated with 1·0 ml. of a 1 : 1 mixture of fuming nitric acid and concentrated sulphuric acid. After the tube had been rotated to wet the surface, it was placed in a boiling water bath for 20 minutes. It was then cooled, 4·0 ml. of distilled water were added and it was cooled again. 2·0 ml. of benzene were now added down the sides of the tube, and small portions of solid

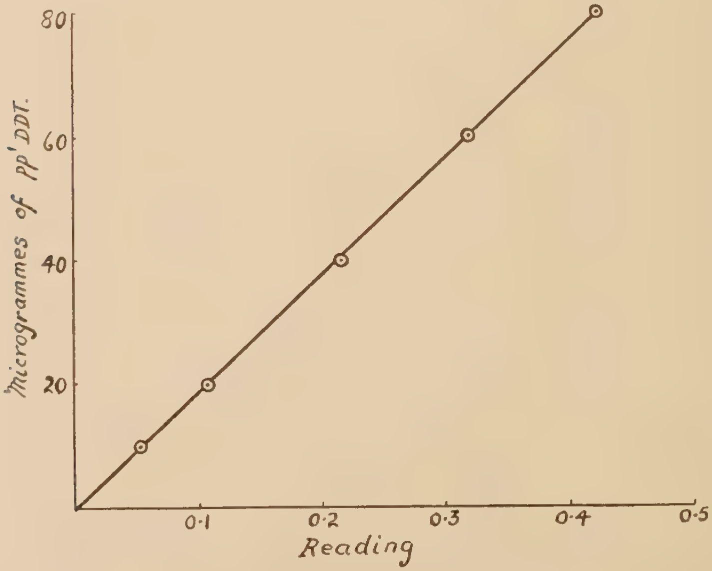

Fig. 2.—Calibration curve for pp'DDT.

Analar sodium carbonate added with gentle shaking until the acids were neutralised, the effervescence serving to transfer the nitrated DDT to the benzene layer. Enough distilled water was next sprayed from a wash bottle down the sides of the tube to remove sodium carbonate clinging to the walls and to bring the benzene layer up to a more accessible position.

By means of a small pipette provided with a rubber teat, the benzene was transferred as completely as possible to a small test tube graduated at 4·0 ml. and 10·0 ml. The nitrated product was more completely recovered by adding 1·0 ml. of benzene down the sides of the nitration tube and transferring as before to the graduated tube. This was repeated once more and the volume of extract finally adjusted to 4·0 ml. A 5·0 per cent. w/v solution of sodium in methanol was added up to the 10·0 ml. mark to develop the colour and the liquids mixed by inversion until optically clear. It was convenient to use tubes with ground glass stoppers in view of the action of sodium methoxide solution on corks.

A Spekker absorptiometer was used in this work to measure the colour developed and readings were made after 10 minutes in 1-cm. cells with Ilford Spectrum filters No. 606.

A calibration curve was constructed by treating known weights of pp′DDT dissolved in benzene in exactly the same way. This was redetermined for each fresh batch of sodium methoxide solution as the amount of colour developed varied with alkali concentration. A typical curve is shown in fig. 2.

Batches of unexposed insects were washed with benzene and the extracts treated in the same way as those from insects exposed to deposits. Mosquitos gave negligible blank colours. Tsetse flies gave a colour absorbing slightly with filter 606. This absorption was allowed for in the results given.

For the chemical procedure, the accuracy is within the limits of ± 5 per cent. as shown by the following recoveries from a series of samples containing a fixed amount of pp′DDT : 41, 43, 40, 41, 40, 42 microgrammes. The accuracy of the complete procedure, including exposure of insects, is given in a succeeding section.

Finally, the intensity of colour developed with pp′DDT was compared with that produced by an equivalent quantity of pure tetra nitro compound (m.p. 223–224°C.) and found to be only slightly less with the filter 606 used. The shade of blue was rather different, being a bright cobalt with the pure nitro compound and nearer a Prussian blue with DDT carried through the analytical procedure described here.

Results.

Weights of insects.

Three-day old female mosquitos, *Aëdes aegypti* (L.), received one blood meal from guinea-pigs several hours before use in tests. The mean weight per insect for batches of 25 mosquitos was 4·89 mg., while the corresponding figure for unfed females of the same age was 2·60 mg.

Tsetse flies, *Glossina palpalis* (R.-D.) used were from 1 to 3 days old and unfed, with a mean weight of 25·4 mg.

Repeatability of Method.

Determinations of the amount of DDT picked up per insect using different numbers of insects showed that results were consistent. Batches of 20 mosquitos were exposed for two-minute contact periods, and tsetse flies were exposed individually for 15 seconds, to deposits from aqueous suspensions of 10–20 micron DDT crystals at a dosage of 25 mg. per sq. ft. on plaster blocks. Results are given in Table I.

Eight replications of 40 mosquitos were exposed for a contact time of 30 seconds to deposits from an aqueous suspension of the DDT crystals at the same dosage

TABLE I.

Species	No. of insects	Mean weight DDT picked up per insect, in microgrammes
A. aegypti ...	20	1·0
	40	1·0
	80	0·8
G. palpalis ...	5	3·2
	10	3·4
	20	3·3

on plaster blocks. The mean weight picked up was 0·48 microgrammes DDT per mosquito with a standard deviation of 0·047.

The repeatability of the method is shown by the following weights picked up from deposits of the DDT crystals by mosquitos in a contact time of two minutes on different days.

17.xi.1950	1·00 μg.
24.xi.1950	1·10, 1·10, 1·05 μg.
14.i.1951	1·00 μg.
16.iii.1951	1·00, 0·95 μg.

Amounts picked up from Plaster Blocks.

Aqueous suspensions of DDT crystals of known size were sprayed at a dosage of 25 mg. DDT per sq. ft. on to series of plaster blocks which were stored for 24 hours at 78°F. and 50 per cent. relative humidity. Batches of 20 mosquitos were then exposed to the blocks for various contact periods. Chemical and biological tests with each particle size range were carried out on the same day. Half the number of mosquitos exposed for each contact period were killed immediately and the amount of DDT they had picked up was determined chemically; the others were transferred to recovery cages and stored for 24 hours before mortality counts were made. Results of these tests are given in Table II.

Mosquitos were active and walked over the treated surface during the exposure period, and the weight of insecticides picked up increased with time. Consideration of the kills in relation to the contact time shows that the 0–10 micron particles are less effective than those in the 10–20 micron range, and above 10 microns there is an inverse relation between particle size and effectiveness. When the amount picked up is taken into account, however, it is found that, weight for weight, the 0–10 micron particles are more effective than the 10–20 micron particles and that there is in fact an inverse relation throughout the range of particle size between particle size and effectiveness.

Probit regression lines with log. (mean weight of DDT picked up per mosquito) as the independent variate were calculated from the data given in Table II. There was no significant difference in the slope of the lines. The median lethal dose increased from 0·12 to 0·42 and 2·13 microgrammes as the particle size range increased from 0–10 to 10–20 and 20–40 microns, respectively.

In the previous paper (Hadaway & Barlow, 1951), it was suggested that the amount of DDT picked up decreased as the particle size increased. The total weight of DDT picked up from deposits of 20–40 micron particles is actually greater than that from deposits of 10–20 micron particles in the same contact period.

TABLE II.

Weights of DDT picked up by mosquitos exposed to crystals at a dosage of 25 mg. per sq. ft. on plaster blocks, and resulting kills 24 hours later. (Eighty mosquitos were used for each determination with 0–10 and 10–20 micron crystals, 40 with 20–40 and 40–60 micron crystals.)

Particle size in microns		Exposure time in minutes							
		0·25	0·5	1	2	4	8	16	32
0–10	Pick-up, µg. DDT per mosquito			0·09	0·13	0·21	0·33		
	Mean percentage kill ...			18	55	93	100		
10–20	Pick-up, µg. DDT per mosquito	0·35	0·48	0·70	1·00				
	Mean percentage kill ...	33	62	90	100				
20–40	Pick-up, µg. DDT per mosquito		0·6	1·1	1·7	2·3	3·05		
	Mean percentage kill ...		0	8	23	50	88		
40–60	Pick-up, µg. DDT per mosquito				0·25	0·5	0·9	1·2	1·3
	Mean percentage kill ...				0	0	0	0	13

Probit-log (weight) regression lines :—

Particle size in microns	Equation	b	LD 50	5% fiducial limits
0–10	$y=6{\cdot}59x-2{\cdot}18.$	$6{\cdot}59 \pm 0{\cdot}94$	0·12 µg.	0·13–0·11 µg.
10–20	$y=6{\cdot}00x-4{\cdot}74.$	$6{\cdot}00 \pm 0{\cdot}64$	0·42 µg.	0·45–0·39 µg.
20–40	$y=5{\cdot}87x-8{\cdot}67.$	$5{\cdot}87 \pm 0{\cdot}87$	2·13 µg.	2·35–1·95 µg.

where y=probit kill

and x=log (dosage picked up in µg. x 10^2).

Similar tests were carried out with tsetse flies exposed for 15 seconds to deposits of DDT crystals at a dosage of 25 mg. per sq. ft. on plaster blocks. Although the numbers of flies were small, the results given in Table III indicate that the relation between particle size, pick-up and toxicity is similar to that shown for mosquitos. The presence of pulvilli and other tarsal differences probably account for the higher dosages picked up by tsetse flies. It is of interest to note that the 0–10 micron particles on plaster are not readily available to tsetse flies although their tarsal structure is so different from that of mosquitos.

TABLE III.

Weights of DDT picked up by tsetse flies exposed for 15 seconds to crystals at a dosage of 25 mg. per sq. ft., and resulting kills 24 hours later.

Particle size in microns	No. of flies	µg. DDT picked up per fly	No. of flies	Mean percentage kill
—	10	0	20	0
0–10	10	0·4	20	40
10–20	10	4·0	20	100
20–40	10	4·6	20	60
40–60	10	2·6	20	10

Retention of particles of different sizes.

Tests were carried out to determine the weights of DDT crystals of different sizes retained after the contact period by mosquitos kept under the same conditions as those in contact toxicity tests. A contact period expected to give approximately 50 per cent. kills after 24 hours was chosen for each particle size range, except the 40–60 micron range. Batches of 20 mosquitos were exposed to deposits from an aqueous suspension of each crystal type at a dosage of 25 mgm. DDT per sq. ft. on plaster blocks. Some were killed at the end of the contact period and the dosage picked up determined chemically. The remainder were transferred to recovery cages and stored at 78°F. for various times before they were killed and the dosage retained by them determined chemically. Some remained in the recovery cages for 24 hours before mortality counts were made in the usual way.

The intervals between exposure to deposits and chemical determinations of the weight of insecticide retained were selected to cover the period until the mosquitos were obviously showing symptoms of DDT poisoning and were beginning to be " knocked down ", *i.e.*, were on the floor of the recovery cage and were showing unco-ordinated movements. It has been noticed throughout these experiments that the rate of action of DDT is related to its particle size. Mosquitos in this test were beginning to be " knocked down " approximately one hour after contact with 0–10 micron particles, two hours with 10–20 micron particles and three hours with 20–40 micron particles, despite the fact that the dosages picked up increased as the particle size increased. It is suggested that solubility of DDT in the cuticle waxes, and therefore penetration to the site of action, becomes more rapid as the crystal size decreases.

The difference between weight picked up and weight retained after a given time may be due to loss from the exterior of the insects by penetration through the cuticle, loss by detachment from the insects during their movements, or both. It appears from the details given in Table IV that the difference between weight picked up and weight retained can be accounted for largely by penetration into the insect in the case of 0–10 and 10–20 micron particles and largely by detachment from the insect in the case of the larger particles. It is well known that particles of below 15 microns in diameter adhere well to insects (Alexander & others, 1944).

Results given in Table IV indicate that effectiveness increases as the particle size decreases even when due allowance is made for the greater proportion of larger particles lost by detachment from the insects. The rate of penetration through the cuticle is obviously an important factor in determining the effectiveness of particles in different size ranges. The fact that DDT can be recovered from the exterior of mosquitos by washing with benzene when they are showing symptoms of DDT poisoning indicates that only a part of the insecticide retained penetrates to the site of action and is responsible for the toxic effects.

TABLE IV.

Retention of DDT particles on mosquitos after contact with deposits from aqueous suspensions at a dosage of 25 mg. DDT per sq. ft. on plaster blocks.

Particle size in microns	Contact time in minutes	Weight of DDT in microgrammes retained per insect after					Mean percentage kill in 24 hours
		0 hr.	0·5 hr.	1 hr.	2 hrs.	3 hrs.	
0–10	2	0·10	0·06	0·05	–	–	49
10–20	0·5	0·5	0·3	0·2	0·2	–	75
20–40	4	2·0	0·6	0·5	–	0·3	52
40–60	16	1·0	0·3	0·2	–	0·1	5

Availability of deposits applied to different materials.

It has been seen that, on plaster blocks, 0–10 micron DDT crystals are less effective than 10–20 micron crystals against mosquitos exposed for the same short contact periods. The reverse is true, however, when aqueous suspensions of crystals in these two size ranges are applied to mud blocks. The mean weight of DDT picked up by 80 mosquitos in 30 seconds from deposits of 25 mg. 0–10 micron DDT crystals per sq. ft. on mud blocks was 0·21 microgrammes per mosquito ; the mean percentage kill of mosquitos exposed for the same contact time was 98 per cent. These figures correspond with those obtained after a contact period of 4 minutes on plaster blocks. Obviously the 0–10 micron DDT crystals are more readily available to mosquitos on mud than on plaster. Adhesion of these small particles to the substrata may be greater on plaster than on mud. Differences in surface irregularities may also affect the availability of these small particles.

Availability of deposits from aqueous suspensions of insecticide particles is influenced considerably by the type of material to which they are applied. When aqueous suspensions are sprayed on to porous materials such as mud and plaster the wetting-agent solution penetrates, to a large extent, into the material and leaves the solid particles fairly freely available on the surface. On non-porous materials, *e.g.* glass, the wetting-agent solution remains on the surface, and, on drying, the wetting-agent solids hold the insecticide particles tenaciously to the material so that they are not readily available for pick-up. Comparison of the results given in Table V with those in Table II shows the difference in availability and effectiveness of deposits from aqueous suspensions of 10–20 micron DDT crystals on glass plates and plaster blocks. Only 0·1 microgramme was picked up per mosquito in 8 minutes on glass compared with 0·48 microgramme in 0·5 minute on plaster.

The importance of the wetting-agent solution in determining the availability of deposits is emphasised by the results obtained with two samples of compressed wallboard. The two samples looked alike but it was found that one (A) wetted fairly easily and the other (B) did so only with difficulty. Deposits from aqueous suspensions of 10–20 micron DDT crystals were more readily available to mosquitos on sample A than on sample B. Dosages picked up from the two samples, and the corresponding kills, are given in Table V. The median lethal dose from wallboard sample A is not significantly different from that for 10–20 micron DDT particles from plaster blocks.

TABLE V.

Weights of DDT picked up by mosquitos exposed to 10–20 micron crystals at a dosage of 25 mg. per sq. ft. on wallboard and glass, and resulting kills in 24 hours. (Forty mosquitos were used for each determination.)

Material		Exposure time in minutes					
		0·25	0·5	1	2	4	8
Wallboard A	Pick-up, μg. DDT per mosquito ...	0·22	0·32	0·48	0·70		
	Mean percentage kill...	5	25	50	85		
Wallboard B	Pick-up, μg. DDT per mosquito ...					0·28	0·35
	Mean percentage kill...			0	3	24	40
Glass ...	Pick-up, μg. DDT per mosquito ...						0·1
	Mean percentage kill...						12

Probit-log (weight) regression line :—

Wallboard A $y=5{\cdot}06\,x-3{\cdot}39$. $b=5{\cdot}06\pm0{\cdot}71$. $LD50=0{\cdot}46\pm0{\cdot}05$.

where y=probit kill.

and $x=\log$ (dosage picked up in μg. DDT per mosquito x 10^2).

Discussion.

McIntosh (1947) investigated the toxicity of types of DDT-crystal suspensions to *Tribolium castaneum* (Hbst.), using a dipping technique, and found that the coarser suspensions were more toxic than the finer ones and that all were more toxic than colloidal DDT. The slope of the probit-log (concentration) regression line for the colloid was different from that for crystalline suspensions. The amounts of DDT retained by the insects at various concentrations of needle aggregates (length c.400μ) and colloidal suspensions were determined and probit-log (weight) regression lines calculated using the linear relationship $W=k\lambda$, where W is the weight of DDT retained on the insects, λ the concentration of DDT in the suspension and k a constant. McIntosh considered the two types as having identical toxicities when compared in this way and concluded that " there are indications that the toxicity of the crystal *in situ* is independent of size, but the evidence for this view is weak."

Stringer (1948) points out that a change of scale based on $W=k\lambda$ will only alter the position of the regression lines and not change the slope, and that a linear logarithmic relationship between W and λ, of the form $W=K\lambda^f$, where f is a retention coefficient, is required to obtain a change of slope. The original regression lines were corrected for retention using the new relationship and it was found that the slopes and the positions of the median lethal dosages were not significantly different. Stringer states that " it appears that the colloid and the needle aggregates are equitoxic when the kill is measured against the amount of DDT acting on the insects."

The two bioassay systems, one involving the dipping of flour beetles in aqueous suspensions of DDT crystals and the other the exposure of mosquitos for short contact periods to dry deposits from aqueous suspensions, are obviously very different and direct comparison of results is not possible.

Attempts have been made in the experiments with mosquitos to relate the response of the test insects measured in terms of the probits of percentage kills to the weights of insecticide acting on these insects rather than to the concentration of the insecticide per unit volume or to the dosage per unit area as is a common practice in bioassay systems. It is clear that several factors are involved in determining the weights of insecticide acting on mosquitos after short contact periods with deposits from aqueous suspensions of DDT particles. The dosage picked up by the mosquitos during the contact period, the extent to which the particles are retained on the insects and the rate at which the insecticide penetrates the cuticle are three of these factors. All are influenced by the particle size of the insecticide and it appears that smaller particles produce a more rapid and greater response.

The availability of particles of any one size range, and therefore the dosage picked up, is influenced considerably by the type of material to which the suspension is applied.

Summary.

The weights of insecticide picked up by mosquitos and tsetse flies in short contact periods on deposits from aqueous suspensions of DDT crystals of different sizes have been determined chemically and correlated with corresponding mortality rates.

The rate of action of DDT particles picked up by mosquitos increases as the size decreases.

Taking into account the weights of insecticide picked up, the median lethal dosage increases as the particle size increases. A further factor, that of retention on the body of the insects, has to be considered, however, for there is evidence that adherence of particles in the 0–10 and 10–20 micron ranges is greater than that of larger particles. Availability, and therefore effectiveness, of deposits from aqueous suspensions of insecticide particles is influenced considerably by the type of material to which they are applied.

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THE IDENTIFICATION OF THE LARVAE OF *ANOPHELES STEPHENSI* LISTON AND *ANOPHELES SUPERPICTUS* GRASSI IN IRAQ.

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In Iraq the normal distribution of *Anopheles superpictus* Grassi is restricted to the desert-steppe and foothill regions north of Lat. 34°N. On the other hand *A. stephensi* Liston is confined to the alluvial plains of the centre and south. However in certain districts, notably in eastern Diyala Liwa, the ranges of the two species overlap. Furthermore, the central plain is subject to occasional small-scale invasion by *A. superpictus*.

The separation of these species in Iraq is therefore of more than merely academic importance. In the adult stage this presents no difficulty but, unfortunately, the larval chaetotaxy is rather similar.

In the past, various authorities have suggested features by which the larvae of these species could be separated, and it is the purpose of this paper to consider these suggestions in the light of the author's experience in Iraq.

Smart (1943) included a key to the larvae of Palaearctic *Anopheles* in which he separates the two species on the degree to which the *posterior clypeal hairs* overlap the bases of the *inner* and *outer clypeal hairs*. In Iraq, the degree of overlap is considerable in both species, often amounting, in *A. stephensi*, to half the total length of the *posterior clypeal hair*.

It may be pertinent to add here, that, in the same key, *A. stephensi* and *A. theobaldi* Giles are separated by the character of the *inner* and *outer clypeal hairs* which, it is claimed, are simple in the former species. In Iraq, however, specimens of *A. stephensi* almost invariably show distinct fraying of the *inner clypeal hairs*. Smart (*loc. cit.*), in his key to the larvae of Oriental *Anopheles* separates these same species in a less dogmatic fashion and one more suited to the Palaearctic key than the rather downright statement it now contains.

In this latter key he follows Puri (1938) in separating the larvae of *A. superpictus* and *A. stephensi* on the relative length of the filament (β) and blade (α) of the *abdominal palmate hairs* (see fig. 1).

During the late war, Major H. S. Leeson, R.A.M.C. (*circa* 1942) produced a pamphlet on the Anopheline mosquitos of the Middle East, with synoptic tables. This author separated the larvae of *A. superpictus* and *A. stephensi* by the relative development of the tubercle of the *inner shoulder hair*, perhaps more widely known as the *inner sub-median prothoracic hair*. This feature has not proved to be reliable for this purpose in Iraq, where the tubercle displays a wide variation in development in both species.

Later in the war (*circa* 1944) Major T. T. Macan, R.A.M.C., revised Leeson's pamphlet and was presumably unable to satisfy himself that a reliable means of differentiation between the larvae of the two species existed. Accordingly, *A. superpictus* and *A. stephensi* were bracketed in the revised larval key, with a note that, in Iraq, their separation is uncertain.

After examination of a large number of larvae of these species in Iraq, it was considered that the only obvious feature of apparent value for the differentiation of *A. superpictus* and *A. stephensi* was the proportionate length of the filament of the *abdominal palmate hairs*. An investigation was then carried out to establish to what extent this method would prove reliable in practice.

A large number of Anopheline larvae from all areas of Iraq are received in this laboratory for routine identification. Many of these are from localities where only one of the species relevant to this paper is known to occur. In such cases, larvae of the *stephensi/superpictus* type are labelled as one or the other species, depending on the place of capture. In most instances, the possibility of erroneous identification is remote. Mature larvae of *A. superpictus* and *A. stephensi* were grouped by this method until a collection of 50 good specimens of each species had been collected. Other than the discarding of early instars, no selection was made. The localities from which the *A. superpictus* were received were Mosul, Shaqlawa, Kirkuk and the bed of the Wand river, near Khanaqin. The localities for the *A. stephensi* larvae were Basrah, Hilla, Kerbela and Baghdad. All specimens were collected between April and July, 1950. They were mounted in chloral gum and allowed to

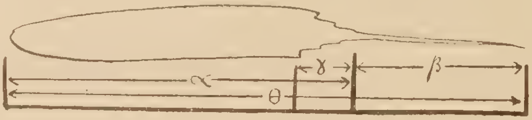

Fig. 1.—Sketch of the leaflet of an abdominal palmate hair ; showing the leaflet (θ), the blade (α), the shoulder (γ), and the filament (β).

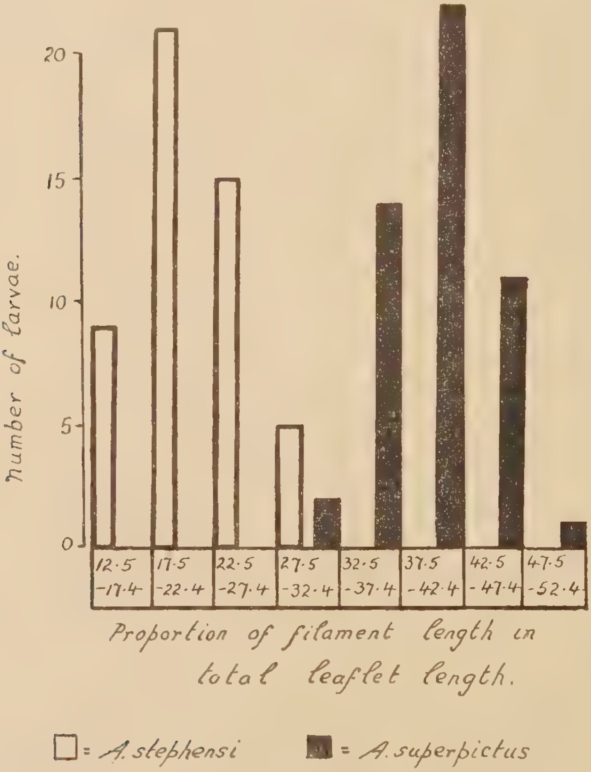

Fig. 2.—Histogram showing frequency distribution of percentage filament lengths to leaflet lengths, *i.e.,* $\frac{\beta \times 100.}{\theta}$

clear before examination. The investigation was limited to the measurement of the length of a leaflet (θ) and its filament (β) of a *palmate hair* on abdominal segment III (see fig. 1). The percentage of leaflet occupied by the filament was calculated in each case.

The results of the measurement of 50 leaflets in each species are presented in the frequency histogram (fig. 2).

It will be noted that the filaments in the *stephensi* larvae were all appreciably less than one-third of the length of the leaflet. The filaments, in the *A. superpictus* larvae on the other hand, were mostly over one-third, or about one-third of this dimension.

In statistical parlance, the " observed distribution " would appear to be " normal " in each species, lending weight to the contention that the original identifications were correct.

However, seven larvae out of the 100 measured fell into a group which was peculiar to neither of the species.

The average absolute measurements of filament and leaflet were as follows :—

	A. stephensi		*A. superpictus*	
	Average length	Standard deviation (σ)	Average length	Standard deviation (σ)
Filament	13 μ	3·66 μ	29 μ	4·6 μ
Leaflet	62 μ	8·85 μ	74 μ	8·4 μ
Average of	$\frac{\beta\times100}{\theta}$: 21 per cent.		$\frac{\beta\times100}{\theta}$: 39 per cent.	

Puri (1931) gives the average measurements of the filaments and blades of the *abdominal palmate hairs* of *A. stephensi* and *A. superpictus* from the Indian region as follows :—

	A. stephensi		*A. superpictus* (India)
	Average length		
	(*a*) Specimens from plains of India	(*b*) Specimens from N.W. Frontier	
Filament	13 μ	26 μ	36·5 μ
Blade	45·8 μ	48 μ	46·7 μ
Leaflet	58·8 μ	74 μ	83·2 μ
Average of	$\frac{\beta\times100}{\theta}$ (*a*) : 23 per cent. (*b*) : 33 per cent.		$\frac{\beta\times100}{\theta}$: 44 per cent.

It is interesting to note that the Iraqi race of *A. stephensi* closely resembles, in these measurements, the specimens from the plains of India rather than those from the N.W. Frontier Province.

In keys to the Oriental Anopheline larvae by Puri (1938) and Smart (1943), the two species are separated according to whether the filament is more or less than half the length of the blade ; in other words with a filament length of more or less than 33·3 per cent. of the leaflet. It would appear that the point of distinction should be altered slightly to be correct for Iraqi specimens. Here, *A. stephensi* larvae should be described as having the filaments " appreciably less than one-third the length of the leaflet." Larvae of *A. superpictus*, on the other hand, should be described as having filaments " about one-third, or more, of the total length of the leaflet ". It is suggested that this will separate the larvae of the two species in over 90 per cent. of the cases.

There are also fairly constant differences in form between the leaflets of the two species. A few examples are sketched in fig. 3.

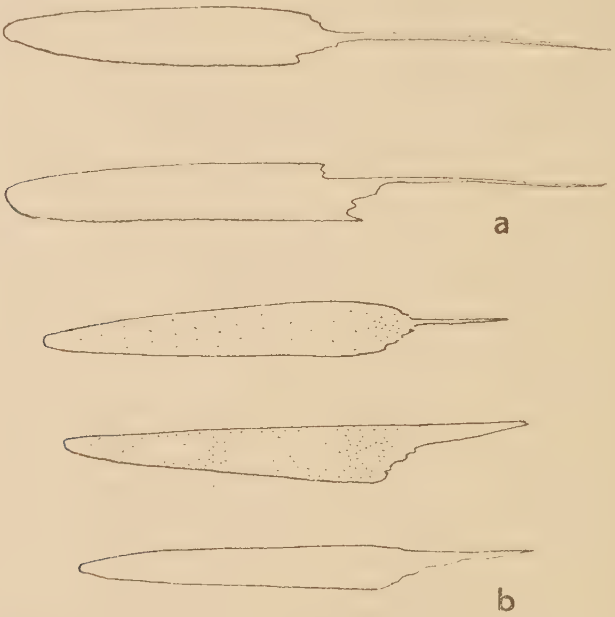

Fig. 3.—Leaflets of palmate hairs of abdominal segment III. (*a*) *A. superpictus* ; (*b*) *A. stephensi*, showing variation commonly encountered.

The leaflets in *A. stephensi* are often deeply and irregularly pigmented ; whereas, in *A. superpictus*, the pigmentation when present is usually lightly and evenly distributed over the hair.

An additional point, of some interest, noticed by Dr. J. H. Cater (personal communication, 1949), is the frequency with which abnormal branching of the *clypeal hairs* is met with in Iraqi specimens of *A. superpictus* larvae. It is usually confined to the *outer clypeal hairs* and is often unilateral. This was noted to be present in 106 out of 332 specimens examined consecutively in 1949. As this abnormality is unknown among larvae of *A. stephensi* in Iraq, its presence in any doubtful larva is suggestive.

Summary.

An investigation on the taxonomy of *A. stephensi* and *A. superpictus* larvae in Iraq is reported upon.

As a result it is suggested that 90 per cent. of the larvae of these species can be separated on the relative length of the filaments of the *abdominal palmate hairs*.

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